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Department of Health, Education, and Welfare
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Proposed Regulations for Tests and Methods of Assay and Certification of Antibiotic Drugs

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Food and Drug Administration

[21 CFR Parts 146, 148e, 148i, 148j, 148o]

ANTIBIOTIC DRUGS

Proposed Regulations for Tests and Methods of Assay and Certification of Antibiotic Drugs Subject to the 1962 Amendments to Section 507 of the Federal Food, Drug, and Cosmetic Act

The Kefauver-Harrison Drug Amendments of 1962 provide that all antibiotic drugs intended for human use shall be subject to the certification provisions of sections 502(1) and 507 of the Federal Food, Drug, and Cosmetic Act. These amendments become effective May 1, 1963. Therefore, the Commissioner of Food and Drugs, pursuant to the provisions of the act (sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357), and under the authority delegated to him by the Secretary of Health, Education, and Welfare (25 F.R. 8625), proposes to issue the following amendments and new regulations to provide for tests and methods of assay and certification of certain antibiotic drugs:

1. It is proposed to amend § 146.1 in the following respects:

a. By adding to paragraph (c) (1) the following new subdivisions:

§ 146.1 Definitions and interpretations applicable to all certifiable antibiotic drugs.

* * * * *

(c) * * *

(1) * * *

(iii) The term "unit" applied to nystatin means the nystatin activity (potency) contained in 0.282 microgram of the nystatin master standard when dried for 2 hours at 40° C. and a pressure of 5 millimeters or less.

(iv) The term "unit" applied to polymyxin B means the polymyxin B activity (potency) contained in 1.272 micrograms of the polymyxin B master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

b. By adding to paragraph (c) (2) the following new subdivisions:

(c) * * *

(2) * * *

(x) The term "microgram" applied to amphomycin means the amphomycin activity (potency) contained in 0.935 microgram of the amphomycin master standard when dried for 4 hours at 60° C. and a pressure of 5 millimeters or less.

(xi) The term "microgram" applied to amphotericin A means the amphotericin A activity (potency) contained in 1.0 microgram of the amphotericin A master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xii) The term "microgram" applied to amphotericin B means the amphotericin B activity (potency) contained in 1.123 micrograms of the amphotericin B master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xiii) The term "microgram" applied to colistin means the colistin activity (potency) contained in 1.51 micrograms of the colistin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xiv) The term "microgram" applied to colistin methanesulfonate means the colistin methanesulfonate activity (potency) contained in 1.93 micrograms of the colistin methanesulfonate master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xv) The term "microgram" applied to cycloserine means the cycloserine activity (potency) contained in 1.0 microgram of the cycloserine master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xvi) The term "microgram" applied to erythromycin means the erythromycin activity (potency) contained in 1.02 micrograms of the erythromycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xvii) The term "microgram" applied to gramicidin means the gramicidin activity (potency) contained in 1.0 microgram of the gramicidin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xviii) The term "microgram" applied to griseofulvin means the griseofulvin activity (potency) contained in 1.0 microgram of the griseofulvin master standard.

(xix) The term "microgram" applied to kanamycin means the kanamycin activity (potency) contained in 1.30 micrograms of the kanamycin master standard.

(xx) The term "microgram" applied to neomycin means the neomycin activity (potency) contained in 1.43 micrograms of the neomycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xxi) The term "microgram" applied to novobiocin means the novobiocin activity (potency) contained in 1.033 micrograms of the novobiocin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

(xxii) The term "microgram" applied to oleandomycin means the oleandomycin activity (potency) contained in 1.176 micrograms of the oleandomycin master standard.

(xxiii) [Reserved]

(xxiv) The term "microgram" applied to oxytetracycline means the oxytetracycline activity (potency) contained in 1.115 micrograms of the oxytetracycline master standard.

(xxv) The term "microgram" applied to paromomycin means the paromomycin activity (potency) contained in 1.333 micrograms of paromomycin master standard.

(xxvi) The term "microgram" applied to tyrothricin means the activity (potency) contained in 0.2 microgram of the gramicidin master standard.

(xxvii) The term "microgram" applied to vancomycin means the vancomycin activity (potency) contained in 1.25 micrograms of the vancomycin master standard.

(xxviii) The term "microgram" applied to viomycin means the viomycin activity (potency) contained in 1.355 micrograms of the viomycin master standard when dried for 3 hours at 60° C. and a pressure of 5 millimeters or less.

c. By adding to paragraph (d) the following new subparagraphs:

(d) * * *

(2) The term "amphotericin A working standard" means a specific lot of a homogeneous preparation of amphotericin A.

(3) The term "amphotericin B working standard" means a specific lot of a homogeneous preparation of amphotericin B.

* * * * *

(12) The term "amphomycin working standard" means a specific lot of homogeneous preparation of amphomycin.

(13) The term "colistin working standard" means a specific lot of a homogeneous preparation of colistin.

(14) The term "colistin methanesulfonate working standard" means a specific lot of a homogeneous preparation of colistin methanesulfonate.

(15) The term "cycloserine working standard" means a specific lot of a homogeneous preparation of cycloserine.

(16) The term "erythromycin working standard" means a specific lot of a homogeneous preparation of erthromycin.

(17) The term "gramicidin working standard" means a specific lot of a homogeneous preparation of gramicidin.

(18) The term "griseofulvin working standard" means a specific lot of a homogeneous preparation of griseofulvin.

(19) The term "kanamycin working standard" means a specific lot of a homogeneous preparation of kanamycin.

(20) The term "neomycin working standard" means a specific lot of a homogeneous preparation of neomycin.

(21) The term "novobiocin working standard" means a specific lot of a homogeneous preparation of novobiocin.

(22) The term "nystatin working standard" means a specific lot of a homogeneous preparation of nystatin.

(23) The term "oleandomycin working standard" means a specific lot of a homogeneous preparation of oleandomycin.

(24) [Reserved]

(25) The term "oxytetracycline working standard" means a specific lot of a homogeneous preparation of oxytetracycline.

(26) The term "paromomycin working standard" means a specific lot of a homogeneous preparation of paromomycin.

(27) The term "polymyxin B working standard" means a specific lot of a homogeneous preparation of polymyxin B.

(28) The term "vancomycin working standard" means a specific lot of a

homogeneous preparation of vancomycin.

(29) The term "viomycin working standard" means a specific lot of a homogeneous preparation of viomycin.

d. By changing paragraph (g) and the introduction to paragraph (h) to read:

(g) Except as specifically provided by the regulations in this chapter, no provision of any regulation shall be construed as exempting any certifiable antibiotic drug from any applicable provision of the act or any regulation thereunder.

(h) The regulations in this chapter, prescribing tests and methods of assay for antibiotic and antibiotic-containing drugs, shall not be construed as preventing the Commissioner from using any other test or method of assay in his investigations to determine whether or not:

2. It is proposed to amend Title 21, Chapter I by adding thereto the following new parts:

PART 148e—ERYTHROMYCIN

Sec.

148e.1 Erythromycin.
148e.2 Erythromycin ethylcarbonate.
148e.3 Erythromycin glucoheptonate.
148e.4 Erythromycin lactobionate.
148e.5 Erythromycin estolate.
148e.6 Erythromycin stearate.
148e.7 Erythromycin ethylsuccinate.
148e.8 Erythromycin sulfate.
148e.9 Erythromycin ethylsuccinate dental powder.
148e.10 Erythromycin for oral suspension.
148e.11 Erythromycin ethylcarbonate for oral suspension.
148e.12 Erythromycin estolate for oral suspension.
148e.13 Erythromycin ethylsuccinate oral suspension.
148e.14 Erythromycin ointment.
148e.15 Erythromycin-neomycin sulfate ointment.
148e.16 Erythromycin-polymyxin B sulfate ointment.
148e.17 Erythromycin estolate capsules.
148e.18 [Reserved]
148e.19 Erythromycin glucoheptonate dental cones.
148e.20 Erythromycin glucoheptonate-polymyxin B sulfate for otic solution.
148e.21 Erythromycin sulfate-polymyxin B sulfate otic solution.
148e.22 Erythromycin estolate for pediatric drops.
148e.23 [Reserved]
148e.24 Erythromycin glucoheptonate for intravenous infusion.
148e.25 Erythromycin lactobionate for intravenous infusion.
148e.26 Erythromycin tablets.
148e.27 Erythromycin stearate tablets.
148e.28 Erythromycin estolate tablets.

AUTHORITY: §§ 148e.1 to 148e.28 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148e.1 Erythromycin.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Erythromycin is the odorless, white to grayish-white or slightly yellow crystalline compound of a kind of erythromycin or a mixture of two or more such compounds. It is so purified and dried that:

(i) It contains not less than 900 micrograms of erythromycin base per milligram calculated on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 10 percent.

(iv) Its pH in a saturated aqueous solution, prepared by using 100 milligrams of erythromycin per milliliter, is not less than 8.0 nor more than 10.5.

(v) It gives a positive identity test for erythromycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing not less than 500 milligrams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay—(1) Potency.* Use either of the following procedures:

(i) *Plate assay—(a) Cylinders (cups).* Use cylinders described under § 141a.1(a) of this chapter.

(b) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. Make nutrient agar as follows:

(1) For carrying the test organism:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Glucose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(2) For base and seed layers: Same formulation as above, except adjust the medium to pH 8.0 after sterilization.

(c) *Working standard.* Dry approximately 30 milligrams of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and dissolve the dried working standard in sufficient methyl alcohol to give a concentration of 10,000 micrograms of erythromycin base per milliliter. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of 1,000 micrograms of erythromycin base per milliliter. This stock solution may be kept under refrigeration for 14 days.

(d) *Preparation of sample.* Dissolve an accurately weighed amount of the sample (approximately 50 milligrams) in approximately 5.0 milliliters of methyl alcohol and further dilute in 0.1*M* potassium phosphate buffer, pH 8.0, to make a solution containing 1.0 milligram of erythromycin base per milliliter (estimated). Further dilute an aliquot to the reference point of the assay with 0.1*M* potassium phosphate buffer, pH 8.

(e) *Preparation of test organism.* The test organism is Sarcina lutea (ATCC 9341), which is maintained on slants of agar described under (b)(1) of this subdivision. Wash the organism from the agar slant with 3 milliliters of sterile sodium chloride solution onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in (b)(1) of this subdivision. Spread the suspension of organism over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 32° C.–35° C., and then wash the resulting growth from the agar surface with about 50 milliliters of sterile sodium chloride solution. Standardize the suspension so that a 1:40 dilution of it will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run test plates to determine the quantity of the bulk suspension (usually 1.5 milliliters) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(f) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in (b)(2) of this subdivision to each Petri dish (20 millimeters×100 millimeters). Distribute the agar evenly on the plates and allow it to harden. Use the plates the same day they are prepared. Melt a sufficient amount of the agar described in (b)(2) of this subdivision, cool to 48° C., add the proper amount of the test organism as described in (e) of this subdivision, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly on the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place six cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(g) *Standard curve.* Prepare daily in 0.1*M* potassium phosphate buffer, pH 8.0, from the stock solution described in (c) of this subdivision, concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 micrograms per milliliter. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution except the 1.0 microgram per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of three plates fill three cylinders with the 1.0 microgram per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 1-microgram determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the point tested for each set of three plates, and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8-microgram concentration, the average of the 36 readings of the 1.0 microgram per milliliter concentration is 16.5 millimeters and the average of the

1.0 microgram per milliliter concentration of this set of three plates is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 0.8 microgram per millimeter concentration of these same three plates is 15.9 millimeters, the corrected value is then 16.1 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on a 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 1.0 microgram per milliliter standard;

a, b, d, e = Corrected average values for 0.64, 0.80, 1.25, and 1.56 micrograms per milliliter solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 1.0 microgram per milliliter solution and three cylinders with the 1.0 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 32° C.–35° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 1.0 microgram per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 1.0 microgram per milliliter value on the curve. From the standard curve, read the potencies corresponding to the corrected values of zone sizes.

(ii) *Spectrophotometric assay* — (*a*) *Reagents.*—(*1*) Alkali reagent: Dissolve 42 grams of tribasic sodium phosphate, dodecahydrate ($Na_3PO_4 \cdot 12H_2O$) in 125 milliliters of 0.5*N* sodium hydroxide, and add 100 milliliters of distilled water. Heat on a steam bath until solution results. Cool and dilute to 250 milliliters with distilled water. Filter before use.

(*2*) 0.05*N* sulfuric acid.

(*3*) 0.05*N* sodium hydroxide.

(*4*) Methyl alcohol (anhydrous), reagent grade.

(*5*) Erythromycin working standard solution: Dry approximately 70 milligrams of erythromycin working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and transfer to a 500-milliliter volumetric flask. Dissolve in 50 milliliters of methyl alcohol, and dilute to 500 milliliters with distilled water. Mix well. This solution may be used for 1 week, if it is kept in a refrigerator.

(*b*) *Apparatus.* A spectrophotometer equipped with a hydrogen lamp source of ultraviolet radiation, and matched, rectangular 1-centimeter silica cells.

(*c*) *Standard erythromycin factor.* Transfer a 10-milliliter aliquot of erythromycin working standard solution to each of four 25-milliliter volumetric flasks. Pipette 1.0 milliliter of 0.05*N* sulfuric acid into two of the flasks, mix well, and set aside at room temperature for 60 minutes. Pipette 2.0 milliliters of alkali reagent into the other two flasks, mix well, and heat in a 60° C. constant temperature water bath for 15 minutes. After heating, transfer the flasks to an ice water bath and cool rapidly to room temperature. Determine the absorbance of these solutions at 236 millimicrons, using distilled water in the reference cell. After the two acid treated solutions have reacted for 60 minutes, pipette 1.0 milliliter of 0.05N sodium hydroxide into each flask. Pipette 20 milliliters of alkali reagent into each flask, mix well, and heat in a 60° C. constant temperature water bath for 15 minutes. Transfer the flasks to an ice water bath and cool rapidly to room temperature. Determine the absorbance of these solutions at 236 millimicrons, using distilled water in the reference cell.

$$\frac{\text{Standard absorbance} - \text{Standard blank absorbance}}{\text{Milligrams of standard}} \times \frac{1{,}000}{\text{Potency of the standard in micrograms per milligram}} = \text{Standard factor K.}$$

Standard factor K = The actual absorbance equivalent to 1 milligram of 100 percent erythromycin base when diluted to a volume of 500 milliliters as outlined in subsection (*a*) of this subdivision.

(*d*) *Samples.* Transfer approximately 100 milligrams of sample, accurately weighed, to a 500-milliliter volumetric flask. Dissolve in 50 milliliters of methyl alcohol and dilute to 500 milliliters with distilled water. Proceed as outlined in (*c*) of this subdivision, except use the following formula:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Sample weight in milligrams}} \times \frac{1{,}000}{\text{Standard factor K}} = \text{Micrograms erythromycin base activity per milligram:}$$

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except administer orally 1.0 milliliter of a suspension of the drug containing 30 milligrams per milliliter in 10-percent acacia solution.

(3) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(4) *pH.* Using a saturated aqueous solution (100 milligrams of erythromycin base per milliliter) proceed as directed in § 141a.5(b) of this chapter.

(5) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(6) *Identity test.* The infrared absorption, in the spectrum from 2 microns to 12 microns, of a 5 percent solution of the sample in chloroform compares qualitatively to that of the erythromycin working standard, similarly treated.

§ 148e.2 Erythromycin ethylcarbonate.

(a) *Requirements for certification*— (1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylcarbonate is a white, crystalline ester of erythromycin obtained by the reaction of a kind of erythromycin and chloroethylcarbonate. It is slightly soluble in water, freely soluble in alcohol, methyl alcohol, acetone, ether, and chloroform. It is so purified and dried that:

(i) It contains not less than 775 micrograms of erythromycin base per milligram, calculated on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 8 percent.

(iv) Its pH is not less than 6.3 and not more than 8.0.

(v) It gives a positive identity test for erythromycin ethylcarbonate.

(2) *Labeling.* It shall be labeled in accordance with the requirements prescribed by § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, identity, and crystallinity.

(ii) Samples required: 10 containers, each containing not less than 500 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following procedures:

(i) *Microbiological plate assay.* Proceed as directed in § 148e.1(b) (1) (i), except to prepare the sample, wash an accurately weighed amount of the sample (approximately 50 milligrams) into a 100-milliliter volumetric flask, using approximately 20 milliliters of absolute methyl alcohol. Immediately dilute to volume with sterile distilled water. Place this solution in a 60° C. water bath for 3 hours or allow it to remain at room temperature for 16 hours to 18 hours to accomplish hydrolysis. Adjust the volume to 100 milliliters with potassium phosphate buffer, pH 8.0, and mix well. Further dilute an aliquot to the reference point of the assay with 0.1*M* potassium phosphate buffer, pH 8.0.

(ii) *Spectrophotometric assay.* Proceed as directed in § 148e.1(b) (1) (ii), except § 148e.1(b) (1) (ii) (*d*). To prepare the sample, transfer approximately 100 milligrams of the accurately weighed sample to a 500-milliliter volumetric flask. Dissolve in 50 milliliters of methyl alcohol, dilute to 500 milliliters with dis-

tilled water, and mix well. Allow the solution to remain at room temperature for 30 minutes to 90 minutes and proceed as directed in § 148e.1(b)(1)(ii)(c), using the following formula to calculate the potency of the sample:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Milligrams of sample}} \times \frac{1{,}000}{\text{Standard factor K}} = \text{Micrograms of erythromycin base activity per milligram.}$$

(2) *Toxicity.* Proceed as directed in § 141d.305(b) of this chapter, except administer orally 0.5 milliliter of a solution containing 100 milligrams (potency) per milliliter in a 10 percent acacia solution.

(3) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a concentration of 200 milligrams per milliliter.

(5) *Identity tests.* Proceed as directed in § 148e.1(b)(6), except that an authentic erythromycin ethylcarbonate is used as the sample of comparison.

(6) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

§ 148e.3 Erythromycin glucoheptonate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin glucoheptonate is the white, crystalline powder of the glucoheptonic acid salt of erythromycin or a mixture of two or more such salts. It is freely soluble in water, alcohol, and methyl alcohol. It is slightly soluble in acetone and chloroform, but is practically insoluble in ether. It is so purified and dried that:

(i) It contains not less than 600 micrograms of erythromycin base per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 5.0 percent.

(vii) Its pH in an aqueous solution containing 25 milligrams per milliliter is not less than 6.0 nor more than 8.0.

(viii) It gives a positive identity test for erythromycin glucoheptonate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, histamine, moisture, pH, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing not less than 500 milligrams.

(*b*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $10.00 for each container in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Use any of the following procedures:

(i) *Microbiological assay.* Proceed as directed in § 148e.1(b)(1)(i).

(ii) *Spectrophotometric assay.* Transfer approximately 100 milligrams of erythromycin glucoheptonate, accurately weighed, to a 500-milliliter volumetric flask. Dissolve with distilled water, add 50 milliliters of methyl alcohol, and dilute to 500 milliliters with distilled water. Mix well, and proceed as directed in § 148e.1(b)(1)(ii)(*c*), using the following calculation:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Sample weight in milligrams}} \times \frac{1{,}000}{\text{Standard factor K}} = \text{Micrograms of erythromycin base per milliliter.}$$

(2) *Sterility.* Use 500 milligrams from each container tested, and proceed as directed in § 141a.2 of this chapter, except that neither the penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using a test dose of 1 milliliter per kilogram of a sterile, pyrogen-free U.S.P. saline test solution containing 50 milligrams of erythromycin base per milliliter.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.5 milliliter of a sterile U.S.P. saline test solution containing 2 milligrams of erythromycin base per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using a test dose of 1 milliliter per kilogram of a sterile saline test solution containing 3 milligrams of erythromycin activity per milliliter.

(6) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a concentration of 25 milligrams per milliliter.

(8) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(9) *Identity.* Proceed as directed in § 148e.1(b)(6), except compare the infrared absorption spectrum with that of an authentic sample of erythromycin glucoheptonate.

§ 148e.4 Erythromycin lactobionate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin lactobionate is the white or slightly yellow, crystalline lactobionate salt of erythromycin. It is freely soluble in water, alcohol, and methyl alcohol. It is slightly soluble in acetone and chloroform, and is practically insoluble in ether. It is so purified and dried that:

(i) It contains not less than 600 micrograms of erythromycin base per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 5 percent.

(vii) Its pH is not less than 6.5 nor more than 7.5.

(viii) It gives a positive identity test for erythromycin lactobionate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, histamine, moisture, pH, crystallinity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $10.00 for each container submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 148e.1(b)(1).

(2) *Sterility.* Using 500 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using as a test dose 1.0 milliliter per kilogram of a sterile, pyrogen-free U.S.P. saline test solution containing 50 milligrams of erythromycin base.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using as a test dose 0.5 milliliter of a sterile U.S.P. saline test solution containing 3 milligrams of erythromycin base per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, administering a test dose of 1 milliliter per kilogram of a sterile distilled water solution containing 3 milligrams per milliliter.

(6) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a concentration of 50 milligrams of erythromycin base per milliliter.

(8) *Identity.* The infrared absorption in the spectrum from 2 microns to

12 microns of a liquid petrolatum dispersion compares qualitatively to that of a similarly prepared authentic erythromycin lactobionate sample.

(9) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

§ 148e.5 Erythromycin estolate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate is the lauryl sulfate salt of the propionyl ester of a kind of erythromycin or a mixture of two or more such salts. It occurs as the white crystalline powder. It is soluble in alcohol, methyl alcohol, acetone, and chloroform, but is practically insoluble in water. It is so purified and dried that:

(i) It contains not less than 600 micrograms of erythromycin base activity per milligram, calculated on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 5 percent.

(iv) Its pH is not less than 4.5 nor more than 7.0.

(v) It gives positive identity tests for erythromycin estolate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, identity, and crystallinity.

(ii) Samples of the batch: A minimum of 10 containers, each containing not less than 500 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subdivision (3) (ii) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Use either of the following procedures:

(i) *Microbiological plate assay*—Proceed as directed in § 148e.2(b)(1)(i), using 1*M* potassium phosphate buffer, pH 8, in lieu of distilled water to prepare the sample.

(ii) *Spectrophotometric assay—*(*a*) *Reagents—*(*1*) Alkali reagent: Dissolve 42 grams of tribasic sodium phosphate, dodecahydrate ($Na_3PO_4 \cdot 12H_2O$) in 125 milliliters of 0.5*N* sodium hydroxide, and add 100 milliliters of distilled water. Heat on a steam bath until solution results. Cool and dilute to 250 milliliters with distilled water. Filter before use.

(*2*) 0.5*N* sulfuric acid.

(*3*) 0.5*N* sodium hydroxide.

(*4*) *Methyl alcohol (anhydrous)*, reagent grade.

(*5*) 0.5 percent potassium phosphate buffer (pH 7.0): Dissolve 13.55 grams of KH_2PO_4 and 27.20 grams of K_2HPO_4 in 5 liters of distilled water. Dilute to 9 liters, and mix well.

(*6*) Erythromycin working standard buffered solution: Dry approximately 70 milligrams of erythromycin working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and transfer to a 500-milliliter volumetric flask. Dissolve in 200 milliliters of methyl alcohol, and dilute to 500 milliliters with 0.5 percent potassium phosphate buffer, pH 7.0. This solution may be used for 1 week if it is kept in a refrigerator.

(*b*) *Apparatus.* A suitable spectrophotometer equipped with a hydrogen lamp source of ultraviolet radiation, and matched, rectangular 1-centimeter silica cells.

(*c*) *Standard erythromycin factor.* Transfer a 10-milliliter aliquot of the erythromycin reference standard buffered solution to each of four 25-milliliter volumetric flasks. Pipette 1.0 milliliter of 0.5*N* sulfuric acid into two of the flasks, mix well, and set aside at room temperature for 60 minutes. Pipette 2.0 milliliters of alkali reagent into the other two flasks, mix well, and heat in a 60° C. constant temperature water bath for 15 minutes. After heating, transfer the flasks to an ice water bath and cool rapidly to room temperature. Dilute to 25 milliliters with distilled water and mix well. Determine the absorbance of these solutions at 236 millimicrons, using distilled water in the reference cell. After the two acid-treated solutions have reacted for 60 minutes, pipette 1.0 milliliter of 0.5*N* sodium hydroxide into each flask. Pipette 2 milliliters of the alkali reagent into the flasks and heat in a 60° C. constant-temperature water bath for 15 minutes. Cool rapidly to room temperature and dilute to 25 milliliters with distilled water. Determine the absorbance of these standard blank solutions at 236 millimicrons, using distilled water in the reference cell.

Calculation:

$$\frac{\text{Standard absorbance} - \text{Standard blank absorbance}}{\text{Milligrams of standard}} \times \frac{1{,}000}{\text{Standard potency}} = \text{Standard factor K.}$$

(*d*) *Sample preparation.* Transfer approximately 100 milligrams of sample, accurately weighed, to a 500-milliliter volumetric flask. Dissolve in 200 milliliters of methyl alcohol, and immediately dilute to 500 milliliters with 0.5 percent potassium phosphate buffer (pH 7.0). Mix well. Proceed as outlined in subsection (*c*) of this subdivision, except use the following formula:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Sample weight in milligrams}} \times \frac{1{,}000}{\text{Standard factor K}} = \text{Micrograms erythromycin base per milligram.}$$

(2) *Toxicity.* Proceed as directed in § 141d.305(b) of this chapter, except to administer orally 0.5 milliliter of a suspension containing 20 milligrams of erythromycin base per milliliter in a 5 percent acacia solution.

(3) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, except prepare the sample in the following manner: Dissolve 1 gram in 5 milliliters of absolute ethyl alcohol and then add 19 milliliters of distilled water.

(5) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(6) *Identity test.* Proceed as directed in § 148e.1(b)(6), except compare the infrared absorption spectrum with that of an authentic sample of erythromycin estolate.

§ 148e.6 Erythromycin stearate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin stearate is the odorless white or slightly yellow crystals or powder of the stearic acid salt of erythromycin. It is practically insoluble in water but is soluble in alcohol, methyl alcohol, chloroform, and ether. It is so purified and dried that:

(i) It contains the equivalent of not less than 500 micrograms of erythromycin base per milligram, calculated on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 5.0 percent.

(iv) Its pH is not less than 7.0 nor more than 10.0.

(v) It gives positive identity tests for erythromycin stearate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, and identity.

(ii) Samples required: A minimum of 10 containers, each consisting of 500 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 148e.1(b)(1)(i), except prepare the sample in the following manner: Dissolve 50 milligrams, accurately weighed, in sufficient absolute methyl alcohol to give a concentration of 1 milligram per milliliter.

(2) *Toxicity.* Proceed as directed in § 141d.305(b) of this chapter, except administer orally 0.5 milliliter of a suspension containing 80 milligrams (potency) per milliliter, prepared by grinding the sample in sterile distilled water, using 1 drop of polysorbate 80 per 3 grams of sample.

(3) *Moisture content.* Proceed as directed in § 141a.26(e) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a 1 percent slurry of erythromycin stearate in water.

(5) *Identity.* Proceed as directed in § 148e.1(b)(6), except compare qualitatively the infrared absorption spectrum with that of an authentic sample of erythromycin stearate.

(6) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

§ 148e.7 Erythromycin ethylsuccinate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate is the white, odorless, crystalline ethylsuccinate salt of erythromycin. It is so purified and dried that:

(i) It contains the equivalent of not less than 765 micrograms of erythromycin base per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) Its moisture content is not more than 2.0 percent.

(v) Its pH is not less than 4.5 and not more than 7.0.

(vi) It gives a positive identity test for erythromycin ethylsuccinate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for Certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) results of tests and assays on the batch for potency, sterility, toxicity, moisture, pH, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) For sterility testing: 10 immediate containers.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3) (ii) (*a*) of this paragraph; $10.00 for all immediate containers submitted in accordance with subparagraph (3) (ii) (*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1 (b) (1) (i), except prepare the sample in the following manner: Dissolve approximately 50 milligrams, accurately weighed, in sufficient absolute methyl alcohol to give a concentration of 1 milligram of erythromycin base per milliliter. Further dilute in 0.1*M* potassium phosphate buffer, pH 8.0.

(2) *Sterility.* Using approximately 500 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141d.305(b) of this chapter, except administer orally 0.5 milliliter of a sterile suspension prepared by grinding the sample with sterile distilled water and 1 drop of polysorbate 80 per 3 grams of sample.

(4) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(5) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a 1.0 percent suspension in water.

(6) *Identity.* Using a suitable spectrophotometer with 0.1-millimeter cells, scan the infrared absorption spectrum between 2 microns and 12.5 microns of a 5 percent chloroform solution of erythromycin ethylsuccinate. The absorption spectrum compares qualitatively to that of an authentic sample of erythromycin ethylsuccinate.

(6) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

§ 148e.8 Erythromycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin sulfate is the sulfate salt of erythromycin. It is an odorless, practically white, crystalline powder. It is so purified and dried that:

(i) It contains the equivalent of not less than 840 milligrams of erythromycin base per milligram, calculated on an anhydrous basis.

(ii) Its moisture content is not more than 7.0 percent.

(iii) Its pH in a solution containing 10 milligrams per milliliter is not less than 4.5 and not more than 6.5.

(iv) It gives a positive identity test for erythromycin sulfate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, and identity.

(ii) Samples required: 10 containers, each consisting of 500 milligrams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1 (b) (1) (i), except prepare the sample in the following manner: Dissolve approximately 50 milligrams, accurately weighed, in sufficient absolute methyl alcohol to give a concentration of 1.0 milligram per milliliter.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *pH.* Proceed as directed in §141 a.5(b) of this chapter, using a concentration of 10 milligrams per milliliter.

(4) *Identity.* Use the following procedures:

(i) *Erythromycin.* Proceed as directed in § 148e.1(b) (1), (6). The infrared absorbtion spectrum compares qualitatively to that of a similarly prepared authentic sample of erythromycin sulfate.

§ 148e.9 Erythromycin ethylsuccinate dental powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate dental powder is a dry mixture of crystalline erythromycin ethylsuccinate, with or without sulfanilamide, and with or without one or more suitable and harmless diluents and flavorings. Each vial contains the equivalent of not less than 10 milligrams of erythromycin base. It is sterile. Its moisture content is not more than 1.0 percent. The crystalline erythromycin ethylsuccinate used conforms to the requirements of § 148e.7(a) (1). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, toxicity, moisture, pH, identity, and crystallinity.

(*b*) The batch for potency, sterility, and moisture.

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 containers, each consisting of 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 30 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One immediate container of each containing 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) (*a*) and (*c*) of this paragraph; $0.75 for each container submitted in accordance with subparagraph (3) (ii) (*b*) (*1*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3) (ii) (*b*) (2) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.1(b) (1), using an appropriate number of immediate containers (usually 10) to prepare the sample for assay. Its potency is satisfactory if it contains the equivalent of not less than 90 percent and not more than 115 percent of the number of milligrams of erythromycin activity that it is represented to contain.

(2) *Sterility.* Use the entire contents of each container tested, and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148e.10 Erythromycin for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin for oral suspension is a dry mixture of erythromycin, with or without suitable and harmless buffer substances, suspending and dispersing agents, diluents, colorings, flavorings, and preservatives. It may contain one or more suitable sulfonamides. The erythromycin content is not less than 40 milligrams of erythromycin base per milliliter of the reconstituted suspension. The erythromycin used conforms to the requirements of § 148e.1(a) (1). Its moisture content is not more than 2 percent. Each other substance used, if its name is recognized in the U.S.P. or N.F. conforms to the

standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, toxicity, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: Six containers, each consisting of 500 milligrams.

(*b*) The batch: A minimum of five immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch, one container each consisting of 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following procedures:

(i) *Microbiological assay.* Proceed as directed in § 148e.1(b) (1) except prepare the sample in the following manner: Reconstitute the drug as directed in the labeling, and dilute an accurately measured aliquot (usually 1.0 milliliter) of the suspension in a 100-milliliter volumetric flask, using a volume-to-volume mixture of 40 parts reagent grade methyl alcohol and 60 parts of 0.1*M* potassium phosphate buffer, pH 8.0.

(ii) *Spectrophotometric assay.* Reconstitute the drug as directed in the labeling, and shake until a uniform suspension is obtained. Proceed as directed in § 148e.1(b) (1) (ii), except prepare the sample for assay as follows: If the solution does not contain a sulfonamide, transfer 2.0 milliliters of the well-shaken suspension to a 1-liter volumetric flask. Add 20 milliliters of methyl alcohol to the flask with swirling to dissolve the erythromycin in the sample. Bring to volume with distilled water, and mix well. If the sample contains a sulfonamide, transfer 2 milliliters of reconstituted suspension to a 125-milliliter separatory funnel. Add 40 milliliters of chloroform and 10 milliliters of water and shake for 1 minute. Collect chloroform extractive in another separatory funnel. Repeat extractions with four 25-milliliter portions of chloroform. Shake the combined chloroform extractives with 10 milliliters of water, and filter the chloroform through a layer of anhydrous sodium sulfate into a 250-milliliter beaker. During the filtration, evaporate the chloroform filtrate in a current of air while warming on a steam bath. Shake the aqueous portion with two 10-milliliter portions of chloroform and wash funnel with chloroform. Evaporate to dryness and dissolve residue in 10 milliliters of methyl alcohol. Transfer the solution to a 1,000-milliliter volumetric flask, and wash beaker with 10-milliliter portions of methyl alcohol. Adjust the volume to 1,000 milliliters with distilled water. Transfer four 10-milliliter aliquots of the final solution to each of four 25-milliliter volumetric flasks. To two flasks add 1 milliliter 0.05*N* H_2SO_4 and set aside at room temperature for 1 hour. To the other two flasks, add 2 milliliters of alkali reagent described in § 148e.1(b) (1) (ii) (*a*) (*1*), and heat in a 60° C. constant temperature bath for 15 minutes. Place the flasks in an ice bath and cool rapidly to room temperature. Dilute to mark with distilled water and determine the absorbance at 236 millimicrons against a sample of distilled water. After the two acid-treated samples have reacted for 1 hour, add 1 milliliter of 0.05*N* NaOH to each flask, add 2 milliliters of alkali reagent described in § 148e.1(b) (1) (ii) (*a*) (*1*), and heat in a 60° C. constant temperature bath for 15 minutes. Place the flasks in an ice bath and cool rapidly to room temperature. Dilute to volume with distilled water and determine the absorbance at 236 millimicrons against a sample of distilled water. Use these absorbance readings as the sample blank. Calculation:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Standard factor K}} = \text{Milligrams erythromycin base per milliliters of reconstituted suspension.}$$

The erythromycin content is satisfactory only if it is not less than 90 percent nor more than 115 percent of label claim.

(2) *Moisture.* Proceed as directed in § 146a.26(e) of this chapter, using the dry powder.

§ 148e.11 Erythromycin ethylcarbonate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ethylcarbonate for oral suspension is a dry mixture of erythromycin ethylcarbonate, with or without suitable and harmless buffer substances, suspending and dispersing agents, diluents, colorings, flavorings, and preservatives. It may contain one or more suitable sulfonamides. The erythromycin ethylcarbonate content is not less than 40 milligrams of erythromycin base per milliliter of the reconstituted suspension. Its moisture content is not more than 2.0 percent. When reconstituted as directed in the labeling, its pH is not less than 6.5 and not more than 8.0. The erythromycin ethylcarbonate used conforms to the requirements of § 148e.2(a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Erythromycin ethylcarbonate used in making the batch for potency, toxicity, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Erythromycin ethylcarbonate used in making the batch: 10 containers, each containing 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One container consisting of 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following procedures:

(i) *Microbiological assay.* Proceed as directed in § 148e.2(b) (1) (i), except prepare the sample in the following manner: Reconstitute the suspension as directed in the labeling. Place 1 milliliter to 5 milliliters of an accurately measured volume of the oral suspension in a 100-milliliter volumetric flask and dissolve in 40 milliliters of reagent grade methyl alcohol. Bring to volume with 0.1*M* potassium phosphate buffer, pH 8.0, and mix well. Hydrolyze either in a 60° C. water bath for 3 hours or keep at room temperature for 16–18 hours. Make subsequent dilutions in 0.1*M* potassium phosphate buffer, pH 8.0.

(ii) *Spectrophotometric assay.* Proceed as directed in § 148e.1(b) (1) (ii), except prepare the sample in the following manner: Reconstitute as directed in the labeling and transfer 5 milliliters of the well-shaken suspension (for the 5-milliliter bottle, pour the entire suspension contents) into a 1,000-milliliter volumetric flask and add 400 milliliters of a 50 percent methyl alcohol-50 percent distilled water solution. Shake until the suspension dissolves, dilute to 1,000 milliliters with distilled water, and mix well. Allow the solution to stand at room temperature for 0.5 hour to 1.5 hours. Further dilute 50 milliliters to 100 milliliters with 10 percent methyl alcohol, and again mix well. Proceed as directed in § 148e.1(b) (1) (ii) (*c*), using the following calculations:

(*a*) Suspension containing more than 5 milliliters per bottle:

$$\frac{\text{(Sample absorbance} - \text{Sample blank absorbance)}}{1.25 \times \text{Factor K}} = \text{Milligrams of erythromycin base per milliliter.}$$

(*b*) Samples containing 5 milliliters or less per bottle:

$$\frac{\text{Sample reading} - \text{Sample blank}}{0.25 \times \text{Factor K}} = \text{Milligrams of erythromycin base per bottle.}$$

The erythromycin ethylcarbonate content is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the suspension prepared as directed in its labeling.

§ 148e.12 Erythromycin estolate for oral suspension.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Erythromycin estolate for oral suspension is a dry mixture of erythromycin estolate, with or without suitable and harmless buffer substances, suspending and dispersing agents, diluents, colorings, flavorings, and preservatives. It may contain one or more suitable sulfonamides. The erythromycin estolate content is not less than 25 milligrams of erythromycin base per milliliter of the reconstituted suspension. When reconstituted as directed in its labelings, its pH is not less than 4.5 and not more than 7.0. Its moisture content is not more than 2.0 percent. The erythromycin estolate used conforms to § 148e.5(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, toxicity, moisture, pH, crystallinity, and identity.

(*b*) The batch: Potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 immediate containers, each consisting of 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One container of each, consisting of 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay—(1) Potency.* Use any of the following procedures.

(i) *Microbiological assay.* Proceed as directed in § 148e.11(b)(1)(i).

(ii) *Spectrophotometric assay—(a) Erythromycin estolate without sulfonamides.* Reconstitute as directed in the labeling, and transfer 5 milliliters of the well-shaken suspension (for the 5-milliliter bottle, pour the entire suspension contents) into a 1,000-milliliter volumetric flask. Add 800 milliliters of a 50 percent mixture of methyl alcohol and the potassium phosphate buffer solution described in § 148e.5(b)(1)(ii)(*a*), and shake to dissolve the erythromycin estolate. Dilute to 1 liter with the methyl alcohol-phosphate buffer solution, and mix well. Hydrolyze the erythromycin estolate in the solution to erythromycin either by keeping it at room temperature overnight (approximately 20 hours), or by keeping the sample in a 60° C. constant-temperature bath for 3 hours. Transfer a 50-milliliter aliquot of the erythromycin solution to a 100-milliliter volumetric flask. Dilute to 100 milliliters with the methyl alcohol-phosphate buffer solution, and mix well. Proceed as directed in § 148e.5(b)(ii)(*c*), using the following calculation:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Standard factor K}} \times \frac{4}{5} = \text{Milligrams of erythromycin base per milliliter of suspension.}$$

The standard factor K is derived as described in § 148e.5(b)(1)(ii)(*c*).

(*b*) *Erythromycin estolate with sulfonamides.* Reconstitute as directed in the labeling, and transfer 2 milliliters of the well-shaken suspension to a 125-milliliter separatory funnel. Add 10 milliliters of water and 40 milliliters of chloroform and then shake for 1 minute. Add 5 milliliters of 10 percent sodium hydroxide solution and shake for 1 minute. Allow the suspension to separate into two layers. Collect the chloroform extractive in a 250-milliliter separatory funnel. Extract the alkaline suspension four more times with 25-milliliter portions of chloroform. Combine the chloroform extractives in the 250-milliliter separatory funnel, and wash the combined extractives with 10 milliliters of water. Filter the washed chloroform through a layer of anhydrous sodium sulfate into a 250-milliliter beaker. Extract the 10 milliliters of wash water with two 10-milliliter portions of chloroform, and add this to the chloroform extractives in the beaker, through the sodium sulfate. Wash the sodium sulfate with chloroform, and collect the washings in the beaker. Evaporate the chloroform to dryness on a steam bath with the aid of a current of air. Dissolve the residue in 10 milliliters of methyl alcohol. Transfer the solution quantitatively to a 500-milliliter volumetric flask. Wash the beaker with four 10-milliliter portions of methyl alcohol and transfer the washes quantitatively to the flask. Add 150 milliliters of methyl alcohol, and mix well. Dilute to 500 milliliters with 0.5 percent potassium phosphate buffer solution (pH 7.0) and mix well. Hydrolyze the erythromycin estolate in the solution to erythromycin by keeping it at room temperature overnight (approximately 20 hours) or by keeping the sample in a 60° C. constant-temperature bath for 180 minutes.

Calculation:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Standard factor K} \times 2.0} = \text{Milligrams of erythromycin base per milliliter of suspension.}$$

The standard factor K is derived as described in § 148e.5(b)(1)(ii)(*c*). The erythromycin estolate content is satisfactory if it is not less than 90 percent nor more than 115 percent of the label claim.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter, using the dry powder.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the drug reconstituted as directed in its labeling.

§ 148e.13 Erythromycin ethylsuccinate oral suspension.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Erythromycin ethylsuccinate oral suspension is erythromycin ethylsuccinate, with or without suitable and harmless buffer substances, suspending and dispersing agents, diluents, colorings, and flavorings, and with or without one or more suitable preservatives. It contains the equivalent of not less than 40 milligrams of erythromycin base per milliliter of the reconstituted suspension. Its moisture content is not more than 5.0 percent. The crystalline erythromycin ethylsuccinate used conforms to the requirements of § 148e.7(a)(1)(i), (iii), (iv), (v), and (vi). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin ethylsuccinate used in making the batch for potency, toxicity, moisture, identity, and crystallinity.

(*b*) The batch for potency and moisture:

(ii) Samples required:

(*a*) The erythromycin ethylsuccinate used in making the batch: 10 containers, each consisting of 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One container of each, consisting of 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in § 148e.11(b)(1)(i). Its potency is satisfactory if it contains not less than 90 percent and

not more than 115 percent of the number of milligrams of erythromycin base that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148e.14 Erythromycin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin ointment is erythromycin, with or without one or more suitable and harmless preservatives and stabilizing agents, and cortisone or a suitable derivative of cortisone, in a suitable and harmless ointment base. Each gram of ointment contains not less than 10 milligrams of erythromycin base, except if it is for ophthalmic use each contains not less than 5 milligrams of erythromycin base. The moisture content is not more than 1.0 percent. The erythromycin used conforms to the standards prescribed by § 148e.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms with the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, pH, moisture, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each immediate container or package submitted in accordance with subparagraph (3)(ii) of this paragraph:

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Microbiological assay.* Proceed as directed in § 148e.1(b)(1)(i), except prepare the sample by either of the following procedures:

(*a*) Place 1 gram of the ointment, accurately weighed, in a 1-liter volumetric flask, add 50 milliliters of peroxide-free ether, and shake until dissolved. Add 200 milliliters of absolute methyl alcohol, and mix thoroughly. Fill to volume and further dilute to the reference point with 0.1*M* potassium phosphate buffer, pH 8.0.

(*b*) Place 1 gram of the ointment, accurately weighed, in a high-speed, glass blending jar with 1 milliliter of polysorbate 80 and 100 milliliters of absolute methyl alcohol and blend for 2 minutes to 3 minutes. Add 399 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0, and blend again for 2 minutes to 3 minutes. Further dilute to the reference point in 0.1*M* potassium phosphate buffer, pH 8.0.

(ii) *Spectrophotometric assay*—(*a*) *Standard procedure.* Dissolve 50 milligrams of erythromycin working standard in 5 milliliters of methyl alcohol and dilute to 50 milliliters with distilled water in a suitable volumetric flask. Transfer 25 milliliters to a 125-milliliter volumetric flask. Add 50 milliliters of petroleum ether, shake the mixture for 1 minute, allow to separate, then draw the aqueous layer through a pledget of cotton into a 200-milliliter volumetric flask. Repeat the extraction of the petroleum ether with two 40-milliliter portions of 80 percent aqueous methyl alcohol, withdrawing each extractive through the cotton into the volumetric flask. Add sufficient anhydrous methyl alcohol to the flask to make a volume of 200 milliliters, and mix well. To each of four 25-milliliter volumetric flasks, add a 5-milliliter aliquot of this solution, plus 5 milliliters of 60 percent aqueous methyl alcohol. To two flasks (blanks) add 1 milliliter of 0.05*N* sulfuric acid, mix well, and set aside at room temperature for 1 hour. At the end of this time, add 1 milliliter of 0.05*N* sodium hydroxide to each of the two flasks, and mix well. To the two untreated flasks (samples) add 2 milliliters of distilled water. Then to all flasks add 2 milliliters of 0.05*N* sodium hydroxide and heat in a 60° C. constant-temperature water bath for 15 minutes. Place the flasks in an ice bath, and cool rapidly to room temperature. Bring the volume in each flask to 25 milliliters with 60 percent aqueous methyl alcohol solution, mix thoroughly, and determine the absorbance at 236 millimicrons, using distilled water from the same source as used in the assay, in the reference cell.

$$\frac{\text{Standard absorbance} - \text{Standard blank absorbance}}{\text{Milligrams of standard}} \times \frac{1{,}000}{\text{Potency of the standard in micrograms per milligram}} = \text{Factor K.}$$

(*b*) *Sample procedure.* Determine two separate samples. Weigh accurately about 2 grams (4 grams, if it is the ophthalmic ointment) of the ointment, and carefully transfer the sample to a 125-milliliter separatory funnel. Add 50 milliliters of petroleum ether and shake to dissolve the ointment base. Extract the erythromycin with three 50-milliliter portions of 80 percent aqueous methyl alcohol. Filter each extractive through a pledget of cotton (previously washed with a few milliliters of the methyl alcohol solution) into a 200-milliliter volumetric flask, add sufficient 80 percent methyl alcohol to make exactly 200 milliliters, and mix well. To each of four 25-milliliter volumetric flasks add a 5-milliliter aliquot of this solution, plus 5 milliliters of 60 percent aqueous methyl alcohol. To two flasks (blanks) add 1 milliliter of 0.05*N* sulfuric acid, mix well, and set aside at room temperature for 1 hour. At the end of this time add 1 milliliter of 0.05*N* sodium hydroxide to each of the two flasks, and mix well. To the two untreated flasks (samples) add 2 milliliters of distilled water. Then to all flasks add 2 milliliters of 0.05*N* sodium hydroxide, and heat in a 60° C. constant temperature bath for 15 minutes. Place the flasks in an ice bath and cool rapidly to room temperature. Bring the volume in each flask to 25 milliliters with 60 percent methyl alcohol, mix thoroughly, and determine the absorbance at 236 millimicrons, using distilled water from the same source as used in the assay in the reference cell.

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Weight of ointment in grams} \times \text{Factor K}} = \text{Milligrams of erythromycin base per gram of ointment.}$$

Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148e.15 Erythromycin-neomycin sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin-neomycin sulfate ointment is erythromycin and neomycin sulfate in a suitable and harmless ointment base. Each gram contains not less than 5 milligrams of erythromycin and the equivalent of not less than 3.5 milligrams of neomycin base. The moisture content is not more than 1 percent. The erythromycin used conforms to the standards prescribed therefor by § 148e.1(a)(1). The neomycin sulfate used conforms to the standards prescribed in § 148i.3(a)(1)(i), (vi), and (vii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, pH, moisture, crystallinity, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, pH, moisture, and identity.

(*c*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3) (ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each container submitted in accordance with subparagraph (3) (ii) (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Erythromycin.* Proceed as directed in § 148e.14(b) (1). Its erythromycin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Neomycin sulfate.* Proceed as directed in § 148i.3(b) (1) of this chapter. Its neomycin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148e.16 Erythromycin-polymyxin B sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin-polymyxin B sulfate ointment is erythromycin and polymyxin B sulfate in a suitable and harmless ointment base. Each gram shall contain not less than 5 milligrams of erythromycin base and not less than 5,000 units of polymyxin B sulfate. The moisture content is not more than 1 percent. The crystalline erythromycin used conforms to the standards prescribed therefor by § 148e.1 (a) (1). The polymyxin B sulfate used conforms to the standards prescribed by § 148p.3(a) (1) (i), (vi), (vii), and (viii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin used in making the batch for potency, pH, moisture, crystallinity, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, pH, moisture, and identity.

(*c*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*c*) The batch: Six immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package, each containing 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3) (ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each container submitted in accordance with subparagraph (3) (ii) (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Erythromycin.* Proceed as directed in § 148e.14(b) (1). The erythromycin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Polymyxin B sulfate.* Proceed as directed in § 148p.3(b) (1) of this chapter. The polymyxin B sulfate content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148e.17 Erythromycin estolate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate capsules are capsules containing erythromycin estolate, with or without suitable and harmless buffer substances and diluents, enclosed in a gelatin capsule. The erythromycin estolate content of each capsule is equivalent to not less than 125 milligrams of erythromycin base. The moisture content is not more than 5 percent. The erythromycin estolate used conforms to the standards prescribed therefor by § 148e.5(a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.3 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, toxicity, pH, moisture, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: Six packages, each containing not less than 500 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(*c*) In case of an initial request for certification, each other ingredient used: One package, each containing not less than 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3) (ii) (*a*) and (*c*) of this paragraph; $0.75 for each capsule submitted in accordance with subparagraph (3) (ii) (*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Microbiological assay.* Proceed as directed in § 148e.2(b) (1) (i), except prepare the sample in the following manner: Using 200 milliliters of reagent-grade methyl alcohol and 300 milliliters of 1*M* potassium phosphate buffer, pH 8.0, blend a representative number of capsules in a high-speed glass blender for 2 to 3 minutes.

(ii) *Spectrophotometric assay.* Transfer four 250-milligram capsules to a 1-liter volumetric flask, or transfer eight 125-milligram capsules to a 1-liter volumetric flask (if necessary, use methyl alcohol to aid in the transfer). Add enough methyl alcohol to make 400 milliliters total. Mix well, and allow to stand for 5 minutes to allow soluble matter to go into solution; then add 0.5 percent potassium phosphate buffer, pH 7.0, mix well, and let stand for 5 minutes. Dilute to 1 liter with 0.5 percent potassium phosphate buffer, pH 7.0, and mix well. Hydrolyze the erythromycin estolate to erythromycin either by keeping it at room temperature overnight (approximately 20 hours) or by keeping the sample in a 60°C. constant-temperature bath for 180 minutes. Filter and discard the first 20 milliliters of the filtrate. Transfer 10 milliliters of the filtrate to a 100-milliliter volumetric flask and fill to volume with 0.5 percent potassium phosphate buffer, pH 7.0, and mixture, in equal parts. Proceed as directed in § 148e.5(b) (1) (ii) (*c*), using the following calculations:

$$\frac{\dfrac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Standard factor K}} \times \dfrac{20}{\text{Number of capsules used}}}{} = \text{Milligrams of erythromycin base per capsule.}$$

Proceed as directed in § 148e.5(b) (1) (ii) (*c*) to derive the standard factor K. Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the equivalent number of milligrams of erythromycin base that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter, using 250 milligrams of the powder.

§ 148e.18 [Reserved]

§ 148e.19 Erythromycin glucoheptonate dental cones.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin glucoheptonate dental cones are composed of erythromycin glucoheptonate, with or without one or more suitable and harmless binders and diluents. The erythromycin glucoheptonate content of each cone is equivalent to not less than 5 milligrams of erythromycin base. The moisture content is not more than 1.5 percent. The crystalline erythromycin glucoheptonate used conforms to the standards prescribed by § 148e.3(a) (1) (i), (iii), (vi), (vii), and (viii). Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standard prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin glucoheptonate used for potency, toxicity, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The erythromycin glucoheptonate used: 10 packages, each containing 500 milligrams.

(b) The batch: A minimum of 30 cones.

(*c*) In case of an initial request for certification, each other ingredient used: One package, each containing 5 grams.

(4) *Fees.* $4.00 for each package submitted in accordance with subparagraph (3) (ii) (a) and (c) of this paragraph; $0.75 for each cone submitted in accordance with subparagraph (3) (ii) (*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following procedures:

(i) *Microbiological assay.* Proceed as directed in § 148e.3(b) (1), except prepare the sample as follows: Blend a representative number of cones in 500 milliliters of 0.1*M*-phosphate buffer, pH 8.

(ii) *Spectrophotometric assay.* Reduce 20 dental cones to a fine powder, transfer the powdered material to a 500-milliliter volumetric flask, and add 400 milliliters of distilled water. Shake, and let stand for about 5 minutes to allow soluble matter to go into solution. Dilute to 500 milliliters with distilled water, and shake well. Proceed as directed in § 148e.1(b) (1) (ii) (*c*), using the following calculation:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Standard factor K} \times \text{Number of dental cones tested}} = \text{Milligrams of erythromycin base per dental cone.}$$

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter:

§ 148e.20 Erythromycin glucoheptonate-polymyxin B sulfate for otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin glucoheptonate-polymyxin B sulfate for otic solution is a packaged combination of one immediate container of dry erythromycin glucoheptonate powder and one immediate container of polymyxin B sulfate in a diluting fluid. It may contain one or more suitable local anesthetic agents and preservatives. When reconstituted as directed in its labeling, each milliliter contains not less than the equivalent of 5 milligrams of erythromycin base and not less than 10,000 units of polymyxin B sulfate. Its pH is not less than 6.0 and not more than 8.0. The moisture content of the dry powder is not more than 5.0 percent. The crystalline erythromycin glucoheptonate used conforms to the requirements of § 148e.3 (a) (1) (i), (vi), (vii), and (viii). The polymyxin B sulfate used conforms to the requirements of § 148p.1(a) (1) (i), (iii), (iv), and (v) of this chapter. Each other ingredient used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin glucoheptonate used in making the batch for potency, moisture, pH, crystallinity, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The batch for potency, pH and for moisture content of the erythromycin glucoheptonate dry powder.

(ii) Samples required:

(*a*) The erythromycin glucoheptonate used in making the batch: 10 packages, each consisting of not less than 500 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each consisting of not less than 500 milligrams.

(*c*) The batch:

(*1*) For all tests except moisture: A minimum of 7 packages.

(2) For moisture content: A minimum of 3 containers.

(*d*) In case of an initial request for certification, each other ingredient used: One package consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each package or container submitted in accordance with subparagraph (3) (ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each package submitted in accordance with subparagraph (3) (ii) (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Erythromycin glucoheptonate*—(*a*) *Microbiological assay.* Proceed as directed in § 148e.3(b) (1) (i), using the drug prepared as directed in its labeling.

(*b*) *Spectrophotometric assay.* Dilute the dry contents of the vial to 250 milliliters with distilled water. Proceed as directed in § 148e.1(b) (1) (ii) (*c*), using the following calculation:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{2 \times \text{Factor K}} = \text{Milligrams of erythromycin base per vial.}$$

The erythromycin glucoheptonate content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Polymyxin B sulfate.* Proceed as directed in § 148p.1(b) (1) of this chapter, using the drug reconstituted as directed in its labeling. The polymyxin B sulfate content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter, using the erythromycin glucoheptonate dry powder.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the reconstituted solution.

§ 148e.21 Erythromycin sulfate-polymyxin B sulfate otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin sulfate-polymyxin B sulfate otic solution is erythromycin sulfate and polymyxin B sulfate, with or without one or more suitable and harmless preservatives and buffers, and with or without one or more suitable local-anesthetic agents, dissolved in a suitable and harmless vehicle. Each milliliter contains the equivalent of not less than 5 milligrams of erythromycin base and not less than 10,000 units of polymyxin B sulfate. Its pH is not less than 5.5 and not more than 8.5. The erythromycin sulfate used conforms to the requirements of § 148e.8(a) (1). The polymyxin B sulfate used conforms to the requirements of § 148p.1(a) (1) (i), (iii), (iv), and (v) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F. conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin sulfate used in making the batch for potency, moisture, pH, identity, and crystallinity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, identity.

(*c*) The batch for potency and pH.

(ii) Samples required:

(*a*) The erythromycin sulfate used in making the batch: 10 packages, each consisting of 500 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each consisting of 500 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: 1 container of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each container submitted in accordanee with subparagraph (3) (ii) (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Erythromycin content.* Proceed as directed in § 148e.1(b) (1) (i), using a 1.0-milliliter aliquot of the solution. The erythromycin sulfate content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams of erythromycin activity that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 148p.1(b)(1) of this chapter, using a 1.0-milliliter aliquot of the solution. The polymyxin B sulfate content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted solution.

§ 148e.22 Erythromycin estolate for pediatric drops.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate for pediatric drops is a dry mixture of erythromycin estolate, with or without suspending and dispersing agents, buffer substances, diluents, colorings, and flavorings. When reconstituted as directed in the labeling, each milliliter contains not less than the equivalent of 100 milligrams of erythromycin base. Its moisture content is not more than 2.0 percent. Its pH is not less than 5.0 nor more than 5.5. The erythromycin estolate used conforms to the requirements prescribed by § 148e.5(a)(1). Each other ingredient used, if its name is recognized in the U.S.P. or the N.F., conforms with the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin estolate used in making the batch for potency, toxicity, pH, moisture, crystallinity, and identity.

(*b*) The batch for potency, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin estolate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch: a minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package, each consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each package or immediate container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Use either of the following procedures:

(i) *Microbiological assay.* Proceed as directed in § 148e.11(b)(1)(i).

(ii) *Spectrophotometric assay.* Reconstitute the suspension as directed in the labeling, and transfer 1 milliliter of the suspension to a 1-liter volumetric flask. Dissolve and dilute the suspension with an equal-parts mixture of absolute alcohol and 0.5 percent potassium phosphate buffer, pH 7.0. Hydrolyze the erythromycin estolate solution to erythromycin by keeping it at room temperature for approximately 20 hours or by keeping the solution in a 60° C. constant-temperature bath for 180 minutes. Proceed as directed in § 148e.5(b)(1)(ii)(*c*), using the following calculations:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Standard factor K}} \times 2 = \text{Milligrams of erythromycin base per milliliter.}$$

The erythromycin estolate potency is satisfactory if it is not less than 90 percent nor more than 115 percent of the amount it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the suspension reconstituted as directed in the labeling.

§ 148e.23 [Reserved]

§ 148e.24 Erythromycin glucoheptonate for intravenous infusion.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin glucoheptonate for intravenus infusion is a dry mixture of erythromycin glucoheptonate and one or more suitable and harmless buffer substances. It contains the equivalent of not less than 250 milligrams of erythromycin activity per vial. It is sterile. It is nontoxic. It is nonpyrogenic. It contains no histamine nor histamine-like substances. Its moisture content is not more than 5 percent. Its pH is not less than 6.0 nor more than 8.0. The erythromycin glucoheptonate used conforms to the standards prescribed by § 148e.3 of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin glucoheptonate used in making the batch for potency, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, toxicity, histamine, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin glucoheptonate used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One container, each containing not less than 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph; $10.00 for each container submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Use either of the following procedures:

(i) *Microbiological assay.* Dissolve the entire contents of an ampul in sufficient absolute methyl alcohol to give a concentration of 10 milligrams of erythromycin base per milliliter. Proceed as directed in § 148e.3(b)(1)(i).

(ii) *Spectrophotometric assay.* Dissolve the entire contents of the ampul in sufficient distilled water to give a concentration of 2 milligrams per milliliter. Dilute further with distilled water to give a concentration of 0.2 milligram per milliliter, and proceed as directed in § 148e.3(b)(1)(ii).

Calculation:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Standard factor K}} \times \frac{\text{Total dilution of sample}}{500} = \text{Milligrams of erythromycin per ampul.}$$

Its potency is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 148e.3(b)(2) of this chapter.

(3) *Pyrogens.* Proceed as directed in § 148e.3(b)(3) of this chapter, using a test dose of 1 milliliter per kilogram of a solution containing 50 milligrams of erythromycin activity per milliliter.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.5 milliliter of a solution in sterile distilled water containing 2 milligrams of erythromycin activity per milliliter.

(5) *Histamine.* Proceed as directed in § 148e.3(b)(5).

(6) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a solution containing 25 milligrams of erythromycin activity per milliliter.

§ 148e.25 Erythromycin lactobionate for intravenous infusion.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Erythromycin lactobionate for intravenous infusion is a dry mixture of erythromycin lactobionate and one or more suitable and harmless buffer substances. It contains the equivalent of not less than 300 milligrams of erythromycin base per vial. It is sterile. It is nonpyrogenic. It is nontoxic. It contains no histamine nor histamine-like substances. Its moisture content is not more than 5 percent. Its pH is not less than 6.5 and not more than 7.5. The erythromycin lactobionate used conforms to the requirements of § 148e.4(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin lactobionate used in making the batch for potency, crystallinity, and identity.

(*b*) The batch for potency, sterility, pyrogens, toxicity, histamine, moisture, and pH.

(ii) Samples required:

(*a*) The erythromycin lactobionate used in making the batch: 10 containers, each consisting of not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One container of each, consisting of not less than 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) (*a*), (*b*) (*1*), and (*c*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3) (ii) (*b*) (2) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Dissolve the entire contents of the vial in sufficient distilled water to give a concentration of 10 milligrams of erythromycin base per milliliter (estimated). Proceed as directed in § 148e.1 (b) (1) (i).

(2) *Sterility.* Proceed as directed in § 148e.4(b) (3).

(3) *Pyrogens.* Proceed as directed in § 148e.4(b) (3).

(4) *Toxicity.* Proceed as directed in § 148e.4(b) (4).

(5) *Histamine.* Proceed as directed in § 148e.4(b) (5).

(6) *Moisture.* Proceed as directed in §141a.26(e) of this chapter.

(7) *pH.* Proceed as directed in § 148e.4(b) (7).

§ 148e.26 Erythromycin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin tablets are erythromycin base, with or without one or more suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings, and with or without one or more suitable sulfonamides. The potency of each tablet is not less than 50 milligrams. Tablets not exceeding 15 millimeters in diameter, or, not intended only for preparing solutions, shall disintegrate within 1 hour. The moisture content is not more than 7.5 percent. The erythromycin base used in making the batch conforms to the requirements of § 148e.1(a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The erythromycin base used in making the batch for potency, toxicity, pH, crystallinity, and identity.

(*b*) The batch for potency, disintegration time, and moisture.

(ii) Samples required:

(*a*) The erythromycin base used in making the batch: Six packages, each containing 500 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: A minimum of 30 tablets.

(*2*) For disintegration time: A minimum of 6 tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: 1 package, each containing not less than 5 grams.

(4) *Fees.* $0.75 for each tablet submitted in accordance with subparagraph (3) (ii) (*b*) (*1*) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3) (ii) (*b*) (2) of this paragraph; $4.00 for each package submitted in accordance with subparagraph (3) (ii) (*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following procedures:

(i) *Microbiological assay.* Proceed as directed in § 148e.1(b) (1) (i), except prepare the sample in the following manner: Blend a representative number of tablets in a high-speed glass blender with absolute methyl alcohol.

(ii) *Spectrophotometric assay*—(*a*) *Erythromycin base.* Place 10 tablets in a 150-milliliter beaker and add 50 milliliters of distilled water. Continue washing and decanting with small portions of distilled water until the colored exterior coating is removed. Allow the tablets to dry, and grind in a mortar. Wash the ground tablets into a 1-liter volumetric flask using 100 milliliters of reagent-grade methyl alcohol and 100 milliliters of distilled water, and fill to volume with distilled water. Filter and dilute 20 milliliters of the filtrate with distilled water to 250 milliliters. Proceed as directed in § 148e.1(b) (1) (ii) (*c*), using the following calculation:

$$2.5\times\frac{\text{Sample absorbance}-\text{Sample blank absorbance}}{\text{Standard factor K}}=\text{Milligrams of erythromycin base per tablet.}$$

(*b*) *Erythromycin base with sulfonamides.* Grind several tablets and weigh an amount of the powder equal to one-half tablet. Transfer the powder to a 125-milliliter separatory funnel; add 25 milliliters of chloroform and 25 milliliters of water and shake for 1 minute. Add 10 milliliters of 10 percent sodium hydroxide solution, and shake for 1 minute. Collect the chloroform extractive in another separatory funnel. Repeat the extraction with four 25-milliliter portions of chloroform. Shake the combined chloroform extractives with 10 milliliters of water, and filter the chloroform through a layer of anhydrous sodium sulfate into a 150-milliliter beaker. Evaporate the chloroform filtrate in a current of air while warming on a steam bath. Shake the aqueous portion with two 10-milliliter portions of chloroform, and wash funnel with chloroform. Evaporate to dryness, and dissolve the residue in 10 milliliters of absolute methyl alcohol. Transfer the solution to a 500-milliliter volumetric flask and wash the residue in the beaker into this volumetric flask with four 10-milliliter portions of absolute methyl alcohol. Adjust the volume to 500 milliliters with distilled water, and proceed as directed in § 148e.1(b) (1) (ii) (*c*), using the following calculation:

$$2\times\frac{\text{Sample absorbance}-\text{Sample blank absorbance}}{\text{Factor K}}=\text{Milligrams of erythromycin base per tablet.}$$

The erythromycin base content is satisfactory if it is not less than 90 percent and not more than 115 percent of the amount it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

§ 148e.27 Erythromycin stearate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin stearate tablets are tablets composed of erythromycin stearate, with or without one or more suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings, and with or without one or more suitable sulfonamides. The potency of each tablet is not less than the equivalent of 50 milligrams of erythromycin base. Tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour. The moisture content is not more than 7 percent. The erythromycin stearate used in making the tablets conforms to the requirements of § 148e.6(a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin stearate used in making the batch for potency, toxicity, pH, crystallinity, and identity.

(b) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(a) The erythromycin stearate used in making the batch: Six containers, each consisting of not less than 500 milligrams.

(b) The batch:

(1) For all tests except disintegration time: A minimum of 30 tablets.

(2) For disintegration time: A minimum of 6 tablets.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package, each containing not less than 5 grams.

(4) *Fees.* $0.75 for each tablet submitted in accordance with subparagraph (3) (ii) (b) (1) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3) (ii) (b) (2) of this paragraph; $4.00 for each package submitted in accordance with subparagraphs (3) (ii) (a) and (c) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148e.26(b) (1) (i).

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

§ 148e.28 Erythromycin estolate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Erythromycin estolate tablets are tablets composed of erythromycin estolate, with or without suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings, and with or without suitable sulfonamides. The potency of each tablet is not less than the equivalent of 50 milligrams of erythromycin base. Tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour. The moisture content is not more than 4.0 percent. The erythromycin estolate used conforms to the requirements of § 148e.5(a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(a) The erythromycin estolate used in making the batch for potency, toxicity, pH, crystallinity, and identity.

(b) The batch for potency, disintegration time, and moisture.

(ii) Samples required:

(a) Erythromycin estolate used in making the batch: Six packages, each containing not less than 500 milligrams.

(b) The batch:

(1) For all tests except disintegration time: A minimum of 30 tablets.

(2) For disintegration time: A minimum of 6 tablets.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package, each containing not less than 5 grams.

(4) *Fees.* $0.75 for each tablet submitted in accordance with subparagraph (3) (ii) (b) (1) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3) (ii) (b) (2) of this paragraph; $4.00 for each package submitted in accordance with subparagraphs (3) (ii) (a) and (c) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following procedures.

(i) *Microbiological assay.* Proceed as directed in § 148e.26(b) (1) (i).

(ii) *Spectrophotometric assay.* Finely powder 10 tablets and accurately weigh a sample equivalent to one-third of a tablet, and transfer to a 125-milliliter separatory funnel. Add 10 milliliters of water and 40 milliliters of chloroform, and then shake for 1 minute. Add 5 milliliters of 10 percent sodium hydroxide solution, and shake for 1 minute. Collect the chloroform extractive in a 250-milliliter separatory funnel. Extract the alkaline suspension four more times with 25-milliliter portions of chloroform. Combine the chloroform extractive in the 250-milliliter separatory funnel, and wash the combined extractives with 10 milliliters of water. Extract the 10 milliliters of wash water with two 10-milliliter portions of chloroform. Filter the combined chloroform extractives through a layer of anhydrous sodium sulfate and evaporate to dryness on a steam bath with the aid of an air current. Dissolve and transfer the residue with five 10-milliliter portions of methyl alcohol to a 500-milliliter volumetric flask. Add methyl alcohol to total 150 milliliters and then fill the flask to volume with 0.5 percent potassium phosphate buffer, pH 7.0. Hydrolyze the erythromycin estolate solution to erythromycin by keeping it at room temperature for 20 hours, or by keeping the solution in a 60° C. constant-temperature bath for 180 minutes. Proceed as directed in § 148e.5(b) (1) (ii) (c), using the following calculation:

$$\frac{\text{Sample absorbance} - \text{Sample blank absorbance}}{\text{Standard factor K}} \times 3 = \text{Milligrams of erythromycin base per tablet.}$$

(2) *Moisture:* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time:* Proceed as directed in § 141a.9(c) of this chapter.

PART 148i—NEOMYCIN SULFATE

Sec.

148i.1 Neomycin sulfate.
148i.2 Neomycin undecylenate.
148i.3 Neomycin sulfate ointment; neomycin sulfate cream.
148i.4 Neomycin sulfate capsules.
148i.5 Neomycin sulfate tablets.
148i.6 Neomycin sulfate oral suspension.
148i.7 Neomycin sulfate nasal suspension; neomycin sulfate nasal solution.
148i.8 Neomycin sulfate otic solution.
148i.9 Neomycin sulfate ophthalmic solution (neomycin sulfate eye and ear drops).
148i.10 Neomycin sulfate lotion; neomycin sulfate foam aerosol.
148i.11 [Reserved]
148i.12 Neomycin sulfate suppositories.
148i.113 Neomycin sulfate for intramuscular injection.
148i.14 Neomycin sulfate surgical powder.
148i.15 Neomycin sulfate-polymyxin B sulfate ophthalmic solution; neomycin sulfate-polymyxin B sulfate ophthalmic solution; neomycin sulfate-polymyxin B sulfate ophthalmic suspension.
148i.16 Neomycin sulfate-polymyxin B sulfate ointment.
148i.17 [Reserved]
148i.18 Neomycin sulfate-polymyxin B sulfate otic solution; neomycin sulfate-polymyxin B sulfate otic suspension.
148i.19 Neomycin sulfate-polymyxin B sulfate vaginal suppositories.
148i.20 Neomycin sulfate-polymyxin B sulfate-gramicidin ophthalmic solution.
148i.21 Neomycin sulfate-polymyxin B sulfate-gramicidin nasal solution.
148i.22 [Reserved]
148i.23 Neomycin sulfate-gramicidin ophthalmic solution; neomycin sulfate-gramicidin ophthalmic suspension.
148i.24 Neomycin sulfate-gramicidin otic solution.
148i.25 Neomycin sulfate-gramicidin lotion.
148i.26 Neomycin sulfate-gramicidin cream; neomycin sulfate-gramicidin ointment.
148i.27 Neomycin sulfate eye or ear ointment.
148i.28 Neomycin sulfate-kaolin-pectin suspension.
148i.29 Neomycin sulfate-nystatin tablets.

AUTHORITY: §§ 148i.1 to 148i.29 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148i.1 Neomycin sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate is the sulfate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) It has a potency of not less than 600 micrograms of neomycin base per milligram.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substance.

(vi) Its moisture content is not more than 8.0 percent.

(vii) Its pH in an aqueous solution containing 33 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(viii) It exhibits absorption maxima, within ±2 millimicrons, at 232 millimicrons and 285 millimicrons when dissolved in sulfuric acid, and the ratio

of the absorption peak at 230 ±2 millimicrons and 285 ±2 millimicrons, $\frac{A_{285}}{A_{232}}$, is not less than 1.20 and not more than 1.50.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, toxicity, histamine, moisture, pH, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $10.00 for each container submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(ii) *Culture media.* Using ingredients that conform to the standards prescribed by the U.S.P. or N.F., make nutrient agar for the seed and base layers:

Beef extract	1.5 gm.
Yeast extract	3.0 gm.
Peptone	6.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.8 to 8.0 after sterilization.

(iii) *Working standard.* Dry the working standard for 3 hours at 60° C. and a pressure of not more than 5 millimeters, and weigh out a sufficient quantity to make a convenient stock solution by diluting with a 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0. This stock solution when stored at a temperature of approximately 15° C. or less may be used for a period not exceeding 1 month.

(iv) *Preparation of sample.* Dissolve volumetrically in 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0, the sample to be tested to make a convenient stock solution. Further dilute volumetrically this solution with 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0, to a final concentration of 10.0 micrograms (estimated) per milliliter, if the test organism is Staphylococcus aureus, or 1.0 microgram per milliliter (estimated) if the test organism is Staphylococcus epidermidis.

(v) *Preparation of test organism.* (*a*) The test organism is Staphylococcus aureus (ATCC 6538P), which is maintained on the agar described in subdivision (ii) of this subparagraph, except that its pH after sterilization is 6.5 to 6.6. From a stock slant, inoculate a Roux bottle containing 300 milliliters of this same agar and incubate for 24 hours at 32° C.–35° C. Wash the resulting growth from the agar surface with about 50 milliliters of sterile sodium chloride solution. Using a suitable photoelectric colorimeter with a filter having a wavelength of 580 millimicrons, adjust the suspension so that a 1:20 dilution gives 25 percent light transmission. Determine by test plates the amount of the bulk suspension to be added to the seed layer to obtain satisfactory zones (usually 0.5 percent). The suspension may be used for 1 week if it is stored under refrigeration.

(*b*) Staphylococcus epidermidis (ATCC 12228), which is maintained on agar described in subdivision (ii) of this subparagraph, may also be used as the test organism. From a stock slant, inoculate a Roux bottle containing this medium and incubate for 24 hours at 32° C.–35° C. Wash the resulting growth from the agar surface, using approximately 50 milliliters of sterile sodium chloride solution, and adjust the suspension so that a 1:25 dilution of it will permit 25 percent light transmission through a filter of 580 millimicrons in a photoelectric colorimeter. Determine by test plates the amount of the bulk suspension to be added to the seed layer to obtain satisfactory zones (usually 2 percent). The suspension may be stored for 1 week under refrigeration.

(vi) *Preparation of plates.* Use plates the same day they are prepared. Evenly distribute 21 milliliters of the melted agar described in subdivision (ii) of this subparagraph to each Petri dish (20 millimeters×100 millimeters); cover with porcelain tops, glazed on the outside, and allow to harden. Evenly spread over the surface of each plate 4.0 milliliters of the same type of melted agar, which has been cooled to approximately 48° C. and inoculated with an organism described in subdivision (v) of this subparagraph. After the seed layer has hardened, place 6 cylinders on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Using the stock working standard solution prepared as in subdivision (iii) of this subparagraph, prepare solutions in 0.1*M* potassium phosphate buffer, pH 8.0, in concentrations of 6.4, 8.0, 10.0, 12.5, and 15.6 micrograms per milliliter if the test organism is Staphylococcus aureus (ATCC 6538P), or in concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 micrograms per milliliter if the test organism is Staphylococcus epidermidis (ATCC 12228). The 10.0 micrograms per milliliter and the 1.0 microgram per milliliter concentrations are used as the reference points. Use a total of 12 plates, 3 plates for each solution except the reference point solution which is included on each plate. On each of the three plates, fill three cylinders with the reference point solution and the other three cylinders with the concentrations under test. Thus, there will be 36 reference point determinations and 9 determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the reference point concentration and the readings of the point tested for each set of three plates and average also all 36 readings of the reference point concentration. The average of the 36 readings of the reference point concentration is the correction point of the curve. Correct the average value obtained for each point to the figure it would be if the reference point reading for that set of three plates were the same as the correction point. Thus, when the Staphylococcus epidermidis method is used, if in correcting the 0.8-microgram concentration, the average of the 36 readings of the 1.0 microgram per milliliter (reference point) concentration is 16.5 millimeters and the average of the 1.0 microgram per milliliter concentration of the set of 3 plates (the 0.8 microgram per milliliter set) is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 0.8 microgram per milliliter concentration of these same three plates is 15.9 millimeters, the corrected value is then 16.1 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the reference point standard solution;

a, b, d, e = Corrected average values for the other standard solutions, lowest to highest concentrations, respectively.

(viii) *Assay procedure.* Use 3 plates for each sample. Fill three cylinders on each plate with the standard and three cylinders with the sample, which has been diluted to the reference point, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 32° C.–35° C., and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the reference point zone of the standard curve. If the average value is lower than the standard value, subtract the difference between them from the reference point value on the curve. From the curve, read the potencies corresponding to these corrected values of zone sizes.

(2) *Sterility.* Using 500 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using 0.5 milliliter of a solution prepared by diluting the sample to approximately 200 micro-

grams activity per milliliter with sterile U.S.P. saline T.S.

(4) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using a test dose of 1.0 milliliter per kilogram of a solution containing 10 milligrams per milliliter in pyrogen-free, sterile saline, T.S.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using as a test dose 0.6 milliliter per kilogram of a solution containing 5 milligrams per milliliter in sterile U.S.P. saline T.S.

(6) *Moisture.* In an atmosphere of about 10 percent relative humidity, transfer about 100 milligrams of the finely powdered sample to a tared weighing bottle equipped with ground-glass top and stopper. Weigh the bottle and place it in a vacuum oven, tilting the stopper on its side so that there is no closure during the drying period. Dry at a temperature of 60° C. and a pressure of 5 millimeters of mercury or less for 3 hours. At the end of the drying period, fill the vacuum oven with air dried by passing it through a drying agent such as sulfuric acid or silica gel. Replace the stopper and place the weighing bottle in a desiccator over a desiccating agent such as phosphorous pentoxide or silica gel, allow to cool to room temperature, and reweigh. Calculate the percent loss.

(7) *pH.* Proceed as directed in § 141a.5 (b) of this chapter, using a solution containing 33 milligrams per milliliter.

(8) *Identity.* Dissolve about 10 milligrams accurately weighed in 1 milliliter of water, add 5 milliliters of an aqueous 40 percent sulfuric acid solution, and heat in a boiling water bath for 100 minutes. Cool and dilute to a volume of 25 milliliters with water. Use a suitable recording ultraviolet spectrophotometer and 1-centimeter quartz cells. Set the instrument to 100 percent transmission with an 8 percent sulfuric acid solution. Record the spectrum from 220 millimicrons to 300 millimicrons. The ratio of the two absorbances $\left(\frac{A_{265}}{A_{232}}\right)$ should be not less than 1.20 and not more than 1.50.

§ 148i.2 Neomycin undecylenate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin undecylenate is the undecylenate salt of a kind of neomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) It contains not less than 300 milligrams of neomycin base activity per milligram, calculated on the anhydrous basis.

(ii) Its moisture content is not more than 5.0 percent.

(iii) The pH of a solution containing 50 milligrams per milliliter in 80 percent methyl alcohol is not less than 6.5 and not more than 7.0.

(iv) It gives positive identity tests for neomycin and undecylenic acid.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(4) *Fees.* $4.00 for each package in the sample submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 148i.1 (b) (1), except prepare the sample for assay as follows: Accurately weigh approximately 500 milligrams of neomycin undecylenate, transfer to a 250-milliliter volumetric flask, add 50 milliliters of chloroform, and mix well. Adjust to volume with absolute alcohol, and mix well. Dilute an aliquot with 0.1*M* potassium phosphate buffer, pH 8.0 to the reference point.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, except use a solution containing 50 milligrams per milliliter in 80 percent methyl alcohol.

(4) *Identity—*(i) *Reagents.* (*a*) Normal sulfuric acid.

(*b*) Chloroform.

(*c*) 0.1*N* potassium permanganate.

(*d*) Freshly distilled aniline.

(*e*) 95 percent ethyl alcohol.

(*f*) Ethyl ether.

(*g*) Activated carbon.

(*h*) 70 percent ethyl alcohol.

(*i*) Normal sodium hydroxide.

(*j*) Triketohydrindene hydrate (Ninhydrin) reagent: Dissolve 200 milligrams of triketohydrindene hydrate in water to make 10 milliliters. Prepare this solution fresh.

(ii) *Procedure.* Suspend 5 grams of neomycin undecylenate in 20 milliliters of normal sulfuric acid. Heat this suspension in a boiling water bath until the liberated undecylenic acid separates on the surface of the aqueous phase. Allow to cool. Transfer the mixture to a separatory funnel and extract it with three 10-milliliter portions of chloroform. Save the aqueous phase in the separator. Filter the combined chloroform extractives through a small pledget of cotton. Heat on a steam bath until the chloroform is completely evaporated. Perform the following tests on the residual liquid:

(*a*) *Undecylenate identity.* (*1*) To about 1 milliliter of the liquid, add potassium permanganate solution dropwise; the permanganate color is discharged.

(*2*) Place about 3 milliliters of the solution and 3 milliliters of freshly distilled aniline in a tall test tube, and heat for 10 minutes at a rate such that the ring of condensate remains just below the mouth of the tube. Cool, add 10 milliliters of alcohol and 10 milliliters of ether, and transfer to a separator. Wash the ether solution with four 20-milliliter portions of water, and discard the water washings. Heat on a steam bath until the odor of ether is no longer perceptible, then add a few milligrams of activated carbon, mix well, and filter. Evaporate the filtrate nearly to dryness, and recrystallize the residue from 70 percent alcohol; the anilide so obtained, after drying, melts between 66° C.–67.5° C.

(*b*) *Neomycin identity.* (*1*) Carefully render just alkaline a 1:10 dilution of the filtrate of the aqueous phase remaining in the separator after chloroform extraction by adding, dropwise, normal sodium hydroxide. Add 1 milliliter of triketohydrindene hydrate reagent; a deep-violet color develops slowly on standing.

(*2*) Add 1 milliliter of sulfuric acid to 1 milliliter of the clear filtrate. Cool, then add 2 drops of a 10 percent solution of α-naphthol in alcohol; a violet color develops on standing. On dilution with 10 milliliters of water, the solution becomes turbid due to the formation of a blue-violet precipitate which dissolves on the addition of 10 milliliters of alcohol, yielding a pale-yellow solution.

§ 148i.3 Neomycin sulfate ointment; neomycin sulfate cream.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate ointment and neomycin sulfate cream is neomycin sulfate in a suitable and harmless ointment or cream base, with or without suitable and harmless buffer substances and dispersing and suspening agents. It may contain cortisone or a suitable derivative of cortisone and one or more suitable proteolytic enzymes Its potency is not less than 3.5 milligrams per gram. If an ointment, its mosture content is not more than 1.0 percent. The neomycin sulfate used conforms to the requirements prescribed by § 148i.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements prescribed by § 148.3 of this chapter, except that, if it is intended solely for topical use and it does not contain cortisone or a derivative of cortisone or a proteolytic enzyme, in lieu of the statement "Caution: Federal law prohibits dispensing without prescription," each package shall include information containing directions and warnings adequate for the use of the drugs by the laity. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages each containing approximately 500 milligrams.

(*b*) The batch: Minimum 5 immediate containers.

(*c*) In the case of an initial request for certification, each other ingredient used in making the batch: One package

of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Extraction (ointment only).* Proceed as directed in § 148i.1(b)(1) of this chapter, except prepare the sample by placing a representative portion of the ointment (usually approximately 1 gram, accurately weighed) into a separatory funnel containing 50 milliliters of peroxide-free ether. Shake the sample and ether until homogeneous. Add 25 milliliters of 0.1*M* potassium phosphate buffer, pH 8.0, and shake well. Allow the layers to separate. Remove the buffer layer and repeat the extraction with new portions of the buffer at least three times and any additional times necessary to insure complete extraction of the antibiotic. Combine the extractives and adjust to an appropriate volume to give a stock solution of convenient concentration. Make further dilutions with 0.1*M* potassium phosphate buffer, pH 8.0.

(ii) *Blending (ointments or creams).* Proceed as directed in § 148i.1(b)(1), except prepare the sample for assay as follows: Prepare an adjusted buffer by making a 1.0 percent solution of 1*N* NaOH in 0.1*M* phosphate buffer, pH 8.0. Transfer an accurately weighed sample into a high-speed glass blender, add 1.0 milliliter of polysorbate 80 and sufficient adjusted phosphate buffer to give a stock solution of convenient concentration. Blend 3 to 5 minutes. The content of neomycin is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* If the product is an ointment, proceed as directed in § 141a.8 (b) of this chapter.

§ 148i.4 Neomycin sulfate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate capsules are capsules composed or neomycin sulfate, with or without one or more suitable and harmless buffer substances, vegetable oils, preservatives, diluents, binders, lubricants, colorings, and flavorings, enclosed in gelatin capsules. Each capsule shall contain not less than 50 milligrams of neomycin sulfate. The moisture content is not more than 4.0 percent. The neomycin sulfate used conforms to the requirements of § 148i.1(a)(1), (i), (iii), (vi), (vii), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum 30 capsules.

(*c*) In the case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each capsule submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph; $4.00 for each immediate container submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148i.1(b)(1), except place a representative number of capsules (usually 5 to 12) in a high-speed glass blender in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to make a stock solution of convenient concentration. Blend for 3 to 5 minutes. Further dilute an aliquot in 0.1*M* potassium phosphate buffer, pH 8.0, to give a solution of 10.0 micrograms (estimated) per milliliter if the test organism is Staphylococcus aureus or to 1.0 microgram (estimated) per milliliter if the test organism is Staphylococcus epidermidis. The content of neomycin is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of activity that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148i.5 Neomycin sulfate tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate tablets are tablets that conform to all requirements and are subject to all procedures prescribed in § 148i.3(a) for neomycin sulfate capsules. In addition to the requirements prescribed by § 148i.3(a), tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: Minimum 30 tablets.

(*2*) For disintegration time studies: Six tablets.

(*c*) In the case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees:* $0.75 for each tablet submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $3.00 for all tablets in the sample submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph; $4.00 for each immediate container submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148i.3(b)(1). Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

§ 148i.6 Neomycin sulfate oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate oral suspension is neomycin sulfate, with or without one or more suitable and harmless suspending and dispersing agents, colorings, flavorings, buffer substances, and preservatives, suspended or dissolved in a suitable and harmless vehicle. It may contain pectin, kaolin, and one or more suitable sulfonamides. Each milliliter shall contain not less than the equivalent of 7.0 milligrams of neomycin base. Its pH is not less than 4.0 and not more than 5.0. The neomycin sulfate used conforms to the requirements of § 148i.1(a)(1) (i), (iii), (vi), (vii), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148i.1(b)(1), except place a representative sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Further dilute in 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 10.0 micrograms (esti-

mated) per milliliter if the test organism is Staphylococcus aureus or 1.0 microgram (estimated) per milliliter if the test organism is staphylococcus epidermidis. Its content of neomycin is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of activity that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted suspension.

§ 148i.7 Neomycin sulfate nasal suspension; neomycin sulfate nasal solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate nasal suspension and neomycin sulfate nasal solution are neomycin sulfate and a suitable vasoconstrictor, with or without one or more suitable and harmless suspending or dispersing agents, preservatives, buffer substances, colorings, and flavorings, and with or without cortisone or a suitable derivative of cortisone, suspended in a suitable and harmless vehicle. Each milliliter contains the equivalent of not less than 3.5 milligrams of neomycin base. The pH is not less than 5.5 and not more than 6.5. The neomycin used conforms to the requirements of § 148i.1(a)(1) (i), (iii), (vi), (vii), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees:* $4.00 for each immediate container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148i.1(b)(1), except dilute volumetrically a representative sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Further dilute an aliquot in 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 10.0 micrograms (estimated) per milliliter if the test organism is Staphylococcus aureus or of 1.0 microgram (estimated) per milliliter if the organism is Staphylococcus epidermidis. Its content of neomycin is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of activity that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted sample.

§ 148i.8 Neomycin sulfate otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate otic solution is neomycin sulfate, with or without one or more suitable and harmless buffer substances and preservatives, suspended or dissolved in a suitable and harmless vehicle. It may contain cortisone or a suitable derivative of cortisone. Each milliliter shall contain the equivalent of not less than 2.5 milligrams of neomycin base. Its pH is not less than 5.5 and not more than 7.5. The neomycin sulfate used conforms to the requirements prescribed by § 148i.1(a)(1) (i), (vi), (vii), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of five immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148i.1(b)(1), except dilute volumetrically a representative sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Further dilute volumetrically in 0.1*M* potassium phosphate buffer, pH 8.0, to give a concentration of 10.0 micrograms (estimated) per milliliter if the test organism is Staphylococcus aureus or 1.0 microgram (estimated) per milliliter if the test organism is Staphylococcus epidermidis. Its content of neomycin is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of activity that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted sample.

§ 148i.9 Neomycin sulfate ophthalmic solution (neomycin sulfate eye and ear drops).

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate ophthalmic solution and neomycin sulfate eye and ear drops is neomycin sulfate, with or without one or more suitable and harmless buffer substances and preservatives, dissolved or suspended in a suitable and harmless vehicle. It may contain cortisone or a suitable derivative of cortisone. Each milliliter shall contain not less than the equivalent of 3.5 milligrams of neomycin base. It is sterile. Its pH is not less than 6.0 and not more than 7.5. The neomycin sulfate used conforms to the requirements prescribed by § 148i.1(a)(1) (i), (ii), (vi), (vii), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for: Potency, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 containers, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) (*a*), (*b*)(*1*), and (3)(ii) (*c*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148i.6(b)(1). Its neomycin content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 141a.2 of this chapter, using the equivalent of approximately 0.5 gram activity, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted sample.

§ 148i.10 Neomycin sulfate lotion; neomycin sulfate foam aerosol.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate lotion or neomycin sulfate foam aerosol is neomycin sulfate suspended or dissolved in a suitable and harmless vehicle, with or without one or more suitable and

harmless suspending agents, colorings, and preservatives. Neomycin sulfate foam is packaged with one or more suitable and harmless inert gases. Both may contain cortisone or a suitable derivative of cortisone. Each gram contains the equivalent of not less than 3.5 milligrams of neomycin base. Its moisture content is not more than 1.0 percent. The neomycin sulfate used conforms to the requirements of § 148i.1(a)(1) (i), (vi), (vii), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 300 milligrams.

(*b*) The batch: Minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in 148i.1 (b) (1), except prepare the sample as follows:

(i) *Neomycin sulfate lotion.* Place a representative sample, accurately measured, into a high-speed glass blender with sufficient volume of 0.1*M* potassium phosphate buffer, pH 8, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Make further dilutions with 0.1*M* potassium phosphate buffer, pH 8.

(ii) *Neomycin sulfate aerosol spray.* Spray the entire contents of the well-shaken sample into a large tared beaker. Place in a vacuum desiccator, evacuate, and allow to stand at room temperature for 16 hours to 18 hours. After the gas has volatilized, accurately weigh the beaker and contents and determine the weight of the material in the beaker. Remove a representative quantity (usually approximately 1 gram, accurately weighed) of the residue and place in sufficient 0.1*M* potassium phosphate buffer, pH 8, to give a stock solution of convenient concentration.

Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148i.12 Neomycin sulfate suppositories.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate suppositories are suppositories composed of neomycin sulfate in a suitable and harmless base, with or without one or more suitable and harmless preservatives. It may contain cortisone or a suitable derivative of cortisone. Each suppository contains the equivalent of not less than 15 milligrams of neomycin base. The moisture content is not more than 1.0 percent. The neomycin sulfate used conforms to the requirements of § 148i.1 (a) (1) (i), (vi), (vii), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium:

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. The expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 30 suppositories.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each suppository in the sample submitted in accordance with subparagraph (3) (ii) (*a*) of this paragraph; $4.00 for each package in the sample submitted in accordance with subparagraph (3) (ii) (*b*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148i.1 (b) (1) of this chapter, except prepare the sample by blending, in a high-speed glass blender, a representative number of suppositories (usually 5 to 12) with 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. The content of neomycin is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 148i.8(b).

§ 148i.13 Neomycin sulfate for intramuscular injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate for intramuscular injection is a dry powder composed of neomycin sulfate, with or without one or more suitable and harmless preservatives, buffer substance, and dispersing agents. Each vial contains the equivalent of not less than 350 milligrams of neomycin base. It is sterile. It is nonpyrogenic. It is nontoxic. It contains no histamine nor histamine-like substances. Its moisture content is not more than 8.0 percent. Its pH in an aqueous solution containing the equivalent of 33 milligrams of neomycin base per milliliter is not less than 5.0 and not more than 7.5. The neomycin sulfate used conforms to the requirements of § 148i.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the requirements prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, pyrogens, toxicity, histamine, moisture, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3) (ii) (*a*), (*b*) (*1*), and (*c*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3) (ii) (*b*) (*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148i.1 (b) (1). Its potency is satisfactory if it contains the equivalent of not less than 90 percent and not more than 115 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 148i.1(b) (2).

(3) *Pyrogens.* Proceed as directed in § 148i.1(b) (4).

(4) *Toxicity.* Proceed as directed in § 148i.1(b) (3).

(5) *Histamine.* Proceed as directed in § 148i.1(b) (5).

(6) *Moisture.* Proceed as directed in § 148i.1(b) (6).

(7) *pH.* Proceed as directed in § 148i.1 (b) (7).

§ 148i.14 Neomycin sulfate surgical powder.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate surgical powder is neomycin sulfate, with or without one or more suitable and harmless diluents, preservatives, and lubricants. Each gram of powder contains

not less than the equivalent of 350 milligrams of neomycin base. It is sterile. Its moisture content is not less than 8.0 percent. The neomycin sulfate used conforms to the requirements prescribed by § 148i.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the requirements prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency, sterility, and moisture.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 5 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) (*a*), (*b*)(*1*), and (*c*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148i.1(b)(1). Its potency is satisfactory if it contains the equivalent of not less than 90 percent and not more than 115 percent of the number of milligrams of neomycin that it is represented to contain.

(2) *Sterility.* Proceed as directed in § 148i.1(b)(2).

(3) *Moisture.* Proceed as directed in § 148i.1(b)(6).

§ 148i.15 Neomycin sulfate-polymyxin B sulfate ophthalmic solution; neomycin sulfate-polymyxin B sulfate ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate ophthalmic solution and neomycin sulfate-polymyxin B sulfate ophthalmic suspension are neomycin sulfate and polymyxin B sulfate, with or without cortisone or a derivative of cortisone, and with or without one or more suitable and harmless preservatives, buffer substances, suspending and wetting agents, and diluents, dissolved or suspended in distilled water. Each milliliter contains not less than 3.5 milligrams of neomycin and not less than 12,500 units of polymyxin. The solution or suspension is sterile. The pH is not less than 5.0 and not more than 7.0. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*c*) The batch for potency, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*c*) The batch for:

(*1*) All tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container or package in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), (*c*)(*1*), and (*d*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*c*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Remove a representative portion from the sample with a suitable syringe, place into an appropriate-sized volumetric flask, and proceed as directed in § 148i.1(b)(1). Its content of neomycin is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Polymyxin content.* Remove a representative portion with a suitable syringe, place in an appropriate-sized volumetric flask and proceed as directed in § 148p.1(b)(1) of this chapter, except calculate from the quantity of neomycin found in the sample, the quantity of neomycin that would be present when the sample is diluted to contain 10 units of polymyxin (labeled potency) per milliliter. Prepare the polymyxin standard curve and the daily standards by adding this quantity of neomycin to each concentration. The content of polymyxin is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(2) *Sterility.* Using the entire contents of one immediate container, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor a control tube is used in the test for bacteria.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted sample.

§ 148i.16 Neomycin sulfate-polymyxin B sulfate ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate ointment is neomycin sulfate and polymyxin B sulfate, with or without cortisone or a suitable derivative of cortisone, and with or without one or more suitable and harmless preservatives, emollients, and suspending and dispersing agents in a suitable and harmless ointment base. Each gram contains not less than 3.5 milligrams of neomycin base and not less than 5,000 units of polymyxin B sulfate. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1)(i), (vi), (vii), and (viii). The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a)(1)(i), (vi), (vii), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The batch for potency and moisture.

(*1*) The number of milligrams of neomycin sulfate per gram of ointment.

(*2*) The number of units of polymyxin B sulfate per gram of ointment.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each immediate container submitted in accordance with subparagraph (3)(ii)(*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 148i.3(b)(1)(ii). Its content of neomycin is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 148p.3(b)(1), except calculate from the quantity of neomycin found when assayed in accordance with subdivision (i) of this subparagraph, the quantity of neomycin that would be present when the sample is diluted to contain 10 units of polymyxin (labeled potency) per milliliter. Prepare the polymyxin standard curve and the polymyxin daily standard by adding this calculated quantity of neomycin to each concentration of polymyxin used for the curve. Use this standard curve to calculate the polymyxin content of the sample. Its content of polymyxin is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

§ 148i.18 Neomycin sulfate-polymyxin B sulfate otic solution; neomycin sulfate-polymyxin B sulfate otic suspension.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate otic solution or neomycin sulfate-polymyxin B sulfate otic suspension is a mixture of neomycin sulfate and polymyxin B sulfate, with or without cortisone and one or more suitable anesthetic agents and antiseptic agents, and with or without one or more suitable and harmless buffer substances and preservatives, suspended or dissolved in a suitable and harmless vehicle. Each milliliter or gram contains not less than 2 milligrams of neomycin base and not less than 4,000 units of polymyxin B sulfate. The pH is not less than 5.5 and not more than 8.5. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1) (i), (vi), (vii), and (viii). The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a)(1) (i), (vi), (vii), and (x) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The batch for neomycin potency, polymyxin B potency, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*c*) The batch: A minimum of 6 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each immediate container submitted in accordance with subparagraph (3)(ii)(*c*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Neomycin content.* Proceed as directed in § 148i.8(b)(1), except that if the vehicle contains liquid petrolatum prepare the sample for assay as follows: Accurately weigh a representative portion (usually approximately 1 gram) and transfer to a high-speed glass blender. Add 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. The content of neomycin is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 148i.17(b)(1), except that if the vehicle contains liquid petrolatum, prepare the sample for assay as follows: Accurately weigh a representative portion (usually 1 gram) and place in a high-speed glass blender. Add 1.0 milliliter of polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Modify the polymyxin standard curve and the daily working standards as follows: Calculate from the quantity of neomycin found in the sample when assayed in accordance with subdivision (i) of this subparagraph, the quantity of neomycin that would be present when the sample is diluted to contain 10 units of polymyxin (labeled potency) per milliliter. Prepare the polymyxin standard curve and the polymyxin daily standard by adding the calculated quantity of neomycin to each concentration of polymyxin used for the curve. Use this standard curve to calculate the polymyxin content of the sample. Its content of polymyxin is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted sample.

§ 148i.19 Neomycin sulfate-polymyxin B sulfate vaginal suppositories.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate vaginal suppositories are suppositories made of neomycin sulfate and polymyxin B sulfate, with or without *N*-acetyl-4-hydroxy-*m*-arsanilic acid, and with or without one or more suitable and harmless surface-acting agents, fillers, binders, lubricants, emollients, dispersing agents, and diluents, in a suitable and harmless base. Each suppository contains the equivalent of not less than 3.5 milligrams of neomycin base activity, and not less than 20,000 units of polymyxin B sulfate. The moisture content is not more than 1.0 percent. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1) (i), (iii), (vi), (vii), and (viii). The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a)(1) (i), (iv), (vi), (vii), and (viii) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*c*) The batch for neomycin potency, polymyxin potency, and moisture.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately equal portions or not less than 500 milligrams.

(*c*) The batch: Not less than 30 suppositories.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), and (*d*) of this paragraph; $1.00 for each suppository submitted in accordance with subparagraph (3)(ii)(*c*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Neomycin content.* Proceed as directed in § 148i.1(b)(1), except prepare the sample for assay as follows: Place a representative number of suppositories (usually 5 to 12) into a high-speed glass blender with 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock solution of convenient concentration. Blend 3 to 5 minutes. The content of neomycin is satisfactory, if it contains not less than 85 percent and not more than 120 percent of the number of milligrams of activity that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 148p.1(b)(1) of this chapter, except prepare the sample for assay as follows: Place a representative number of suppositories (usually 5 to 12) into a high-speed glass blender, and add 1.0 milliliter of polysorbate 80 and sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a stock solution of convenient concentration. Modify the polymyxin standard curve as follows: Calculate from the quantity of neomycin found when assayed in accordance with subdivision (i) of this subparagraph, the

quantity of neomycin that would be present when the sample is diluted to contain 10 units of polymyxin (labeled potency) per milliliter. Prepare the polymyxin standard curve and the polymyxin daily standards by adding this calculated quantity of neomycin to each concentration of polymyxin used for the curve. Use this standard curve to calculate the polymyxin content of the sample. Its content of polymyxin is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148i.20 Neomycin sulfate-polymyxin B sulfate-gramicidin ophthalmic solution.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-gramicidin ophthalmic solution is neomycin sulfate, polymyxin B sulfate, and gramicidin, with or without one or more suitable and harmless dispersing and suspending agents, buffer substances, diluents, surfactants, and antiseptics, dissolved in a suitable and harmless vehicle. Each milliliter contains not less than 1.75 milligrams of neomycin base activity, not less than 5,000 units of polymyxin B sulfate, and not less than 0.025 milligram of gramicidin. It is sterile. Its pH is not less than 5.0 and not more than 6.0. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1). The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a)(1) of this chapter. The gramicidin used conforms to the standards prescribed by § 148f.1(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, toxicity, moisture, crystallinity, and identity.

(*d*) The batch for neomycin potency, polymyxin potency, gramicidin potency, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*d*) The batch:

(*1*) For all tests except sterility: Minimum of 7 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*e*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), (*c*), and (*e*) of this paragraph; $6.00 for each immediate container submitted in accordance with subparagraph (3)(ii) (*d*)(*1*) of this paragraph; $10.00 for all immediate containers submitted in accordance with subparagraph (3)(ii)(*d*)(*2*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Neomycin content.* Proceed as directed in § 148i.15(b)(1)(i). The neomycin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 148i.15(b)(1)(ii). The polymyxin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(iii) *Gramicidin content.* Proceed as directed in § 148f.1(b)(1) of this chapter, except to prepare the sample for assay remove a representative sample with a suitable syringe, place into an appropriate-sized volumetric flask, and dilute with 95 percent ethyl alcohol to give a stock solution of convenient concentration. The gramicidin content is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(2) *Sterility.* Using the entire contents from each immediate container, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted solution.

§ 148i.21 Neomycin sulfate-polymyxin B sulfate-gramicidin nasal solution.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-polymyxin B sulfate-gramicidin nasal solution is a solution of neomycin sulfate, polymyxin B sulfate, and gramicidin, with or without cortisone or a suitable derivative of cortisone, with or without a suitable vasoconstrictor and local anesthetic, and with or without one or more suitable and harmless buffer substance, diluents, surfactants, and anti-inflammatory agents, dissolved in distilled water. Each milliliter contains not less than the equivalent of 0.6 milligram of neomycin base activity, 2,000 units of polymyxin B sulfate, and 0.05 milligram of gramicidin. The pH is not less than 5.0 and not more than 6.5. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1) (i), (iv), (vi), (vii), and (viii). The polymyxin B sulfate used conforms to the standards prescribed by § 148p.1(a)(1) (i), (iii), (vi), (vii), and (viii) of this chapter. The gramicidin used conforms to the standards prescribed by § 148f.1(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The polymyxin B sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*c*) The gramicidin used in making the batch for potency, toxicity, moisture, crystallinity, and identity.

(*d*) The batch for neomycin potency, polymyxin potency, gramicidin potency, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing not less than approximately 500 milligrams.

(*b*) The polymyxin B sulfate used in making the batch: 10 packages, each containing not less than approximately 500 milligrams.

(*c*) The gramicidin used in making the batch: 10 packages, each containing not less than 500 milligrams.

(*d*) The batch: A minimum of 7 immediate containers.

(*e*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), (*c*), and (*e*) of this paragraph; $6.00 for each immediate container submitted in accordance with subparagraph (3)(ii)(*d*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Neomycin content.* Proceed as directed in § 148i.20(b)(1)(i). Its content of neomycin is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Polymyxin content.* Proceed as directed in § 148i.15(b)(1)(ii). Its content of polymyxin B sulfate is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of units that it is represented to contain.

(iii) *Gramicidin content.* Proceed as directed in § 148f.1(b)(1) of this chapter, except to prepare the sample for assay remove a representative sample with a suitable syringe, place into an ap-

propriate-sized volumetric flask and dilute with 95 percent ethyl alcohol to give a stock solution of convenient concentration. Its content of gramicidin is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted sample.

§ 148i.23 Neomycin sulfate-gramicidin ophthalmic solution; neomycin sulfate-gramicidin ophthalmic suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-gramicidin ophthalmic solution or neomycin sulfate-gramicidin ophthalmic suspension is neomycin sulfate and gramicidin, with or without cortisone or a suitable derivative of cortisone, and one or more suitable antiseptics and vasoconstrictors, and with or without one or more suitable and harmless buffer substances, suspending agents, dispersing agents, surfactants, preservatives, and diluents, dissolved or suspended in sterile distilled water. Each milliliter contains the equivalent of not less than 0.66 milligram of neomycin base and not less than 0.025 milligram of gramicidin activity. It is sterile. The pH is not less than 6.0 and not more than 7.4. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1). The gramicidin used conforms to the standards prescribed by § 148f.1(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by each official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, toxicity, moisture, crystallinity, and identity.

(*c*) The batch for neomycin potency, gramicidin potency, sterility, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The gramicidin used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*c*) The batch:

(*1*) For all tests except sterility: A minimum of 6 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii)(*a*), (*b*), and (*d*) of this paragraph; $5.00 for each immediate container submitted in accordance with subparagraph (3)(ii)(*c*)(*1*) of this paragraph; $10.00 for all immediate containers submitted in accordance with subparagraph (3)(ii)(*c*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 148i.15(b)(1)(i), using an aliquot of 1.0 milliliter of the sample. The neomycin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Gramicidin content.* Proceed as directed in § 148f.1(b)(1) of this chapter, except to prepare the sample for assay place a 1.0-milliliter aliquot of the sample in an appropriate sized volumetric flask and dilute with 95 percent ethyl alcohol to give a stock solution of convenient concentration. The content of gramicidin is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(2) *Sterility.* Using the entire contents of an immediate container, proceed as directed in § 148a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted sample.

§ 148i.24 Neomycin sulfate-gramicidin otic solution.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-gramicidin otic solution is a solution of neomycin sulfate and gramicidin, with or without cortisone or a suitable derivative of cortisone, with or without one or more suitable local antiseptics and antifungal agents, and with or without one or more suitable and harmless surfactants, buffer substances, preservatives, and diluents, dissolved in distilled water. Each milliliter contains the equivalent of not less than 0.66 milligram of neomycin base and not less than 0.05 milligram of gramicidin activity. The pH is not less than 5.0 and not more than 6.0. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1)(i), (vi), (vii), and (viii). The gramicidin used conforms to the standards prescribed by § 148f.1(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, moisture, crystallinity, and identity.

(*c*) The batch for neomycin potency, gramicidin potency, and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The gramicidin used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*c*) The batch: Seven immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii)(*a*), (*b*), and (*d*) of this paragraph; $5.00 for each immediate container submitted in accordance with subparagraph (3)(ii)(*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 148i.15(b)(1)(i). The neomycin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Gramicidin content.* Proceed as directed in § 148f.1(b)(1) of this chapter, except to prepare the sample for assay, remove a representative sample with a suitable syringe, place in an appropriate-sized volumetric flask, and dilute with 95 percent ethyl alcohol to give a stock solution of convenient concentration. The gramicidin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted solution.

§ 148i.25 Neomycin sulfate-gramicidin lotion.

(a) *Requirements for certification*—(1)—*Standards of identity, strength, quality and purity.* Neomycin sulfate-gramicidin lotion is neomycin sulfate and gramicidin, with or without cortisone or a suitable derivative of cortisone, with or without one or more antiseptics, and with or without one or more suitable and harmless buffer substances, surfactants, dispersing, suspending, and preservative agents, and diluents in a suitable and harmless lotion base. Each milliliter contains the equivalent of not less than 2.5 milligrams of neomycin base and not less than 0.25 milligram of gramicidin activity. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1)(i), (vi), (vii), and (viii). The gramicidin used conforms to the standards prescribed by § 148f.1(a)(1) of this chapter, except § 148f.1(a)(1)(ii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the requirements prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3

of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, moisture, crystallinity, and identity.

(*c*) The batch for neomycin potency and gramicidin potency.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: Seven packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The gramicidin used in making the batch: Seven packages, each containing approximately equal portions of not less than 500 milligrams.

(*c*) The batch: A minimum of 7 immediate containers.

(*d*) In case of an initial request for certification, each other substance used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3) (ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each immediate container submitted in accordance with subparagraph (3) (ii) (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 148i.10(b) (1) (i). Its neomycin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Gramicidin content.* Proceed as directed in § 141f.1(b) (1) of this chapter, except prepare the sample by placing an accurately measured representative portion into a high-speed glass blender with sufficient 95 percent alcohol to give a stock solution of convenient concentration. Blend 3 to 5 minutes. Its gramicidin content is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

§ 148i.26 Neomycin sulfate-gramicidin cream; neomycin sulfate-gramicidin ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-gramicidin cream of neomycin sulfate-gramicidin ointment is neomycin sulfate and gramicidin, with or without cortisone or a suitable derivative of cortisone, and with or without one or more suitable and harmless suspending and dispersing agents, preservatives, perfuming agents, coloring agents, and buffer substances, in a suitable and harmless cream or ointment base. Each gram contains the equivalent of not less than 2.5 milligrams of neomycin base and not less than 0.25 milligram of gramicidin activity. If it is an ointment, the moisture content is not more than 1.0 percent. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a) (1) (i), (iii), (vi), (vii), and (viii). The gramicidin used conforms to the standards prescribed by § 148f.1(a) (1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* Its label and labeling shall comply with the requirements of § 148.3 of this chapter, except that if the drug is not for ophthalmic use or does not contain cortisone or a suitable derivative of cortisone, in lieu of the statement "Caution: Federal law prohibits dispensing without prescription," the labeling of each package shall include information containing directions and warnings adequate for use of the drug by the laity. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The gramicidin used in making the batch for potency, moisture, crystallinity, and identity.

(*c*) The batch for neomycin potency, gramicidin potency, and moisture, if it is an ointment.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: Seven packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The gramicidin used in making the batch: Seven packages, each containing approximately equal portions of not less than 500 milligrams.

(*c*) The batch: Minimum of 7 immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3) (ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each immediate container submitted in accordance with subparagraph (3) (ii) (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Proceed as directed in § 148i.3(b) (1) (i) or (ii). Its content of neomycin is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Gramicidin content*—(*a*) *Cream.* Proceed as directed in § 148f.1(b) (1) of this chapter, except prepare the sample for assay by placing an accurately weighed representative portion (usually 1 gram) into a high-speed glass blender with 1.0 milliliter of polysorbate 80 and sufficient 95 percent alcohol to give a stock solution of convenient concentration. Blend 3 to 5 minutes.

(*b*) *Ointment.* Proceed as directed in § 148f.1(b) (1) of this chapter, except prepare the sample for assay by placing an accurately weighed representative sample (usually approximately 1 gram) into a separatory funnel. Dissolve the ointment in 50 milliliters of *n*-hexane. Extract this solution with four 50-milliliter portions of 95 percent ethyl alcohol. Collect the extractives in a 250-milliliter volumetric flask, bring to volume with 90 percent ethyl alcohol, and mix well. From this stock solution, dilute an aliquot with 95 percent ethyl alcohol to reference concentration.

Its content of gramicidin is satisfactory if it is not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* If the preparation is an ointment, proceed as directed in § 141a.8(b) of this chapter.

§ 148i.27 Neomycin sulfate eye or ear ointment.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate eye-ear ointment is neomycin sulfate, with or without cortisone or a suitable derivative of cortisone, in a suitable and harmless ointment base. Each gram contains the equivalent of not less than 3.5 milligrams of neomycin as the base. Its moisture content is not more than 1.0 percent. The neomycin sulfate used conforms to the requirements of § 148i.1 (a) (1) (i), (vi), (vii), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The neomycin sulfate used: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each consisting of approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148i.3 (b) (1) (i) or (ii). Its content of neomycin is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.8(b) of this chapter.

§ 148i.28 Neomycin sulfate-kaolin-pectin suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-kaolin-pectin suspension is neomycin sulfate, kaolin, and pectin, with or without one or more suitable and harmless suspending and dispersing agents, buffer substances, flavorings, colorings, and preservatives, suspended in distilled water. Each milliliter contains the equivalent of 7.0 milligrams of neomycin base. The pH is not less than 3.8 and not more than 4.8. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1) (i), (iii), (vi), (vii), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The batch for neomycin potency and pH.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The batch: A minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each consisting of approximately 5 grams.

(4) *Fees.* $4.00 for each package or each immediate container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in 148i.1(b)(1) of this chapter, except prepare the sample for assay as follows: Accurately weigh not less than approximately 1 gram of the sample, transfer to a 250-milliliter volumetric flask, dissolve and dilute to mark with 0.1*M* potassium phosphate buffer, pH 8.0. Further dilute with 0.1*M* potassium phosphate buffer, pH 8.0, to 10 micrograms (estimated) per milliliter. The content of neomycin is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter.

§ 148i.29 Neomycin sulfate-nystatin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Neomycin sulfate-nystatin tablets are neomycin sulfate and nystatin with a suitable and harmless binder and lubricating substance. Each tablet contains the equivalent of not less than 0.35 gram of neomycin base and not less than 125,000 units of nystatin. The moisture content is not more than 10 percent. Tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1) (i), (iii), (vi), (vii), and (viii). The nystatin used conforms to the standards prescribed by § 148k.1(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The neomycin sulfate used in making the batch for potency, toxicity, moisture, pH, and identity.

(*b*) The nystatin used in making the batch for potency, toxicity, moisture, pH, and identity.

(*c*) The batch for neomycin potency, nystatin potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The nystatin used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*c*) The batch for:

(*1*) All tests except disintegration time: A minimum of 30 tablets.

(*2*) For disintegration-time studies: Six tablets.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each consisting of approximately 5 grams.

(4) *Fees.* $1.00 for each tablet submitted in accordance with subparagraph (3)(ii)(*c*)(*1*) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3)(ii)(*c*)(*2*) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), and (*d*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Neomycin content.* Using not less than three tablets, proceed as directed in § 148i.5(b)(1).

(ii) *Nystatin content.* Proceed as directed in § 148k.7(b)(1) of this chapter, using not less than three tablets for preparing the sample.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

PART 148j—NOVOBIOCIN

Sec.

148j.1 Sodium novobiocin.
148j.2 Calcium novobiocin.
148j.3 Sodium novobiocin tablets.
148j.4 Calcium novobiocin tablets.
148j.5 Calcium novobiocin syrup.
148j.6 [Reserved]

AUTHORITY: §§ 148j.1 to 148j.6 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148j.1 Sodium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin is the crystalline monosodium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 800 micrograms per milligram, expressed in terms of the novobiocin acid on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 6.0 percent.

(vii) Its pH in a solution containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(viii) When dissolved in 0.1*M* sodium borate, it will demonstrate a positive color identity test for novobiocin with a saturated aqueous solution of *N*,2,6-trichloroquinoneimine.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, toxicity, histamine, moisture, pH, crystallinity, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $10.00 for each container submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(ii) *Culture media.* Use ingredients that conform to the standards prescribed in the U.S.P. or N.F., if such name appears in either compendium.

(*a*) Make nutrient agar for the seed layer and for carrying the test organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Glucose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s	1,000 ml.

pH 6.5 to 6.6 after sterilization.

(*b*) Make nutrient agar for the base layer as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000 ml.

pH 6.5 to 6.6 after sterilization.

In lieu of preparing the media from the individual ingredients specified in this subdivision, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media.

(iii) *Working standard.* Dissolve volumetrically a suitable weighed quantity (30 milligrams to 50 milligrams) of the working standard, dried as described in § 141a.5(a) of this chapter, in 2 milliliters of ethyl alcohol and then add sufficient 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0, to give a concentration of 1,000 micrograms of novobiocin acid per milliliter. This stock solution may be kept for 30 days when stored under refrigeration.

(iv) *Preparation of sample.* Dissolve in sufficient 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0, to give a convenient stock solution. Further dilute in 1.0 percent potassium phosphate buffer pH 6.0, to give a final concentration of 0.5 microgram of novobiocin acid per milliliter (estimated).

(v) *Preparation of test organism.* The test organism is Staphylococcus epidermidis (ATCC 12228), which is maintained on slants of agar described in subdivision (ii) (*a*) of this subparagraph. Wash the organisms from a recently incubated slant with 3 milliliters of sterile sodium chloride solution onto a large agar surface, such as that provided by a Roux bottle containing 300 milliliters of agar. Use sterile glass beads to aid in spreading the organisms over the entire agar surface of the Roux bottle. Incubate 16 hours to 24 hours at 32° C. to 35° C. Wash the resulting growth from the agar surface with 50 milliliters of sterile sodium chloride solution. Standardize the resulting bulk suspension so that a 1:25 dilution in sodium chloride solution will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run test plates to determine the quantity of the bulk suspension (usually 1.0 milliliter) to be added to each 100 milliliters of agar that will give clear, sharp zones of inhibition of appropriate size.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in subdivision (ii) (*b*) of this subparagraph to each Petri dish (20 millimiters×100 millimeters). Distribute the agar evenly in the plates, cover with porcelain tops glazed on the outside, and allow to harden. Use plates the same day they are prepared. Melt a sufficient amount of agar described in subdivision (ii) (*a*) of this subparagraph, cool to 48° C., add the proper amount of the test organism bulk suspension as described in subdivision (v) of this subparagraph, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the seed agar evenly in the plates, cover again, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 micrograms per milliliter stock solution in 1 percent potassium phosphate buffer, pH 6.0, to obtain concentrations of 0.32, 0.40, 0.50, 0.63, and 0.78 microgram of novobiocin acid per milliliter. Use three plates for the determination of each point on the curve, except the 0.5 microgram per milliliter concentration, a total of 12 plates. On each of three plates, fill alternately three cylinders with the 0.5 microgram per milliliter standard, and fill the other three cylinders with the concentration under test. Thus, there will be thirty-six 0.5-microgram determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 0.5 microgram per milliliter concentration and the readings of the point tested for each of three plates, and average also all 36 readings of the 0.5 microgram per milliliter concentrations. The average of the 36 readings of the 0.5 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 0.5 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.4-microgram concentration, the average of the 36 readings of the 0.5-microgram concentration is 20.0 millimeters, and the average of the 0.5-microgram concentration of this set of three plates is 19.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 0.4-microgram concentration of these same three plates is 19.0 millimeters, the corrected value is 19.2 millimeters. Plot these corrected values, including the average of the 0.5 microgram per milliliter concentration, on two-cycle semilog paper, placing the concentration in micrograms per milliliter on the logarithmic scale and the diameter of the zone of inhibition on the arithmetic scale. Draw the standard curve through these points either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Corrected zone diameter for the lowest concentration of standard curve;

H = Corrected zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 0.5 microgram per milliliter standard;

a, b, d, e = Corrected average values for the 0.32, 0.40, 0.63, and 0.78 microgram per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 0.5 microgram per milliliter solution and three cylinders with the 0.5 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 32° C.–35° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 0.5 microgram per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 0.5 microgram per milliliter value on the standard curve. From the curve, read the potencies corresponding to these corrected values of zone sizes.

(2) *Sterility.* Using 500 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using as a test dose 1.0 milliliter per kilogram of a solution prepared by diluting the sample to approximately 10 milligrams of activity per milliliter with sterile, pyrogen-free distilled water.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except use sterile U.S.P. saline T.S. as a diluent, and administer a test dose of 0.5 milliliter of a solution containing 4 milligrams of novobiocin acid per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using a test dose of 0.6 milliliter per kilogram of an aqueous solution containing 3 milligrams per milliliter.

(6) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a saturated aqueous solution prepared by mixing well 25 milligrams of novobiocin acid per milliliter of distilled water.

(8) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(9) *Identity.* (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 20 micrograms (approximate) of novobiocin acid per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 1.0 milliliter of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *n*-butanol and shake well. A green color should develop in the

n-butanol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should be developed in the benzene layer.

§ 148j.2 Calcium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin is the crystalline calcium salt of a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 800 micrograms per milligram, expressed in terms of novobiocin acid on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 10 percent.

(iv) Its pH in a saturated aqueous suspension of 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(v) When dissolved in 0.1*M* sodium borate it will demonstrate a positive color identity test for novobiocin with saturated aqueous solution of *N*,2,6-trichloroquinoneimine.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, crystallinity, and identity.

(ii) Samples required: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3) (ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148j.1 (b) (1), except prepare the stock solution of the sample by dissolving and diluting with absolute ethyl alcohol to an estimated concentration of 1,000 micrograms per milliliter. The solution for assay is diluted with 1 percent potassium phosphate buffer, pH 6.0, to an estimated concentration of 0.5 microgram per milliliter.

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except use sterile U.S.P. saline T.S. as the diluent, and inject 0.5 milliliter of a solution containing the equivalent of 2 milligrams of novobiocin acid per milliliter.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a saturated aqueous solution prepared by suspending 25 milligrams of calcium novobiocin per milliliter.

(5) *Identity.* (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 20 micrograms (approximate) novobiocin acid per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle, 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 1.0 milliliter of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well, and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *n*-butanol, and shake well. A green color should develop in the *n*-butanol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.) and shake well. A pink color should be developed in the benzene layer.

§ 148j.3 Sodium novobiocin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin tablets are tablets that contain sodium novobiocin, with or without one or more suitable and harmless buffer substances, diluents, binders, lubricants, colorings, and flavorings, and with or without one or more suitable sulfonamides. Each tablet contains not less than 125 milligrams of novobiocin. Its moisture content is not more than 3 percent. Tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall distintegrate within 1 hour. The crystalline sodium novobiocin used conforms to the requirements of § 148j.1 (a) (1) (i), (iv), (vi), (vii), and (viii). Each other substance used, if its name is recognized in the U.S.P. or N.F., shall conform to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Novobiocin sodium used in making the batch for potency, toxicity, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency, moisture, disintegration time.

(ii) Samples required:

(*a*) Novobiocin sodium used in making the batch: 10 packages, each containing equal portions of approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: Minimum of 30 tablets.

(*2*) For disintegration time studies: Six tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each tablet in the sample submitted in accordance with subparagraph (3) (ii) (*b*) (*1*) of this paragraph; $3.00 for all tablets in the sample submitted in accordance with subparagraph (3) (ii) (*b*) (*2*) of this paragraph; $4.00 for each immediate container submitted in accordance with subparagraph (3) (ii) (*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148j.1 (b) (1), except prepare the sample by placing a representative number of tablets (usually 5 to 12) in a high-speed glass blender with sufficient 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0, to give a stock solution of convenient concentration. The average content of novobiocin is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams that each is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter. The tablets are satisfactory if they disintegrate within 1 hour.

§ 148j.4 Calcium novobiocin tablets.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin tablets are tablets composed of calcium novobiocin, with or without one or more suitable sulfonamides and with or without one or more suitable harmless colorings, binders, flavorings, diluents, lubricants, and buffer substances. Each tablet contains not less than 125 milligrams of novobiocin. Its moisture content is not more than 4.0 percent. Tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour. The novobiocin calcium used meets the requirements of § 148j.2(a) (1) of this chapter, except § 148j.2(a) (1) (ii), (iii), and (v). Each other substance used, if its name appears in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The calcium novobiocin used in making the batch for potency, toxicity, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) The calcium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: Minimum of 30 tablets.

(*2*) For disintegration time: Six tablets.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing not less than approximately 5 grams.

(4) *Fees.* $4.00 for each container in the samples submitted in accordance with subparagraph (3) (ii) (*a*) and (*c*) of this paragraph; $0.75 for each tablet submitted in accordance with subparagraph (3) (ii) (*b*) (*1*) of this paragraph; and $3.00 for all tablets submitted in accordance with subparagraph (3) (ii) (*b*) (*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Blend a representative number of tables (usually 5 to 12) in a high-speed glass blender for 3 to 5 minutes

with sufficient absolute ethyl alcohol to give a stock solution of convenient concentration. Proceed further as directed in § 148j.2(b)(1). The average content of novobiocin is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter, using not less than three tablets ground to a fine powder.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter.

§ 148j.5 Calcium novobiocin syrup.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Calcium novobiocin syrup is a syrup prepared from calcium novobiocin, with or without one or more suitable sulfonamides and with or without one or more suitable and harmless buffer substances, suspending and stabilizing agents, preservatives, coloring, and flavoring substances suspended or dissolved in a suitable and harmless vehicle. Each milliliter contains not less than 25 milligrams of novobiocin. The pH is not less than 6.0 and not more than 7.0. The calcium novobiocin used conforms to the requirements of § 148j.2(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The calcium novobiocin used in making the batch for potency, toxicity, moisture, pH, crystallinity, and identity.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The calcium novobiocin used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 5 immediate containers.

(c) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Remove a representative sample of the syrup with a suitable syringe, place into a high-speed glass blender with sufficient absolute ethyl alcohol to give a concentration (estimated) of 1,000 micrograms per milliliter. Blend for 3 to 5 minutes. Proceed further as directed in § 148j.2(b)(1). Its novobiocin content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted syrup.

PART 148o—PAROMOMYCIN

Sec.
148o.1 Paromomycin sulfate.
148o.2 Paromomycin sulfate capsules.
148o.3 Paromomycin sulfate syrup.

AUTHORITY: §§ 148o.1 to 148o.3 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148o.1 Paromomycin sulfate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality and purity.* Paromomycin sulfate is the sulfate salt of a kind of paromomycin or a mixture or two or more such salts. It is a creamy-white to light-yellow powder. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms per milligram on an anhydrous basis.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 5.0 percent.

(iv) The pH in a 3.0 percent aqueous solution is not less than 5.0 and not more than 7.5.

(v) Its specific rotation at 25° C. in water is not less than +50° and not more than +55° on an anhydrous basis.

(vi) Its ash content is not more than 2.0 percent.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, specific rotation, and ash content.

(ii) Samples of the batch: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Plate assay using Staphylococcus epidermidis—*(*a*) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(*b*) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(*1*) Make nutrient agar for maintaining the test organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Glucose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(2) Use the nutrient agar described in this subsection for the seed layer and base layer, except that its pH after sterilization is 7.8 to 8.0.

(*c*) *Working standard.* Dry approximately 30 milligrams of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the weight of the dried standard and dissolve in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a concentration of 1,000 micrograms of paromomycin per milliliter. This stock solution may be kept in the refrigerator for 3 weeks.

(*d*) *Preparation of sample.* Dissolve the sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a convenient stock solution. Further dilute in 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 1.0 microgram per milliliter (estimated).

(*e*) *Preparation of test organism.* The test organism is *Staphylococcus epidermidis* (ATCC 12228), which is maintained on slants of agar described in (*b*)(*1*) of this subdivision. Wash the organism from the agar slant with 3 milliliters of sterile sodium chloride solution onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in (*b*)(*1*) of this subdivision. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 32° C., and then wash the resulting growth from the agar surface with 50 milliliters of sterile sodium chloride solution. Standardize the suspension, so that a 1:25 dilution will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run plates to determine the quantity of the bulk suspension (usually 0.16 milliliter) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(*f*) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in (*b*)(2) of this subdivision to each Petri dish (20 millimeters×100 millimeters). Distribute the agar evenly in the plates and allow it to harden. Use plates the same day they are prepared. Melt a sufficient amount of agar described in (*b*)(2) of this subdivision, cool to 48° C., add the proper amount of the test organism as described in (*e*) of this subdivision, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, cover, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*g*) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 micrograms per milliliter stock solution in 0.1*M* potassium phosphate buffer, pH 8.0, to obtain concentrations of 0.64, 0.80, 1.00, 1.25, and 1.56 micrograms per milliliter. The 1.0-microgram per milliliter solution is the reference concentration. Use three plates for the determination of each point on the curve, except the 1.0-microgram per milliliter concentration (a total of 12 plates). On each of three plates fill three cylinders with the 1.0-microgram per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 1-microgram determinations and 9 determinations for each of the other points on the curve. After incubation, read the diameters of the circles of inhibition in the plates. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the

point tested for each set of three plates, and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point of the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.80-microgram concentration, the average of 36 readings of the 1.0-microgram concentration were 20.0 millimeters, and the average of the 1.0-microgram concentration of this set of three plates were 19.8 millimeters, the correction would be +0.2 millimeter. If the average reading of the 0.80-microgram concentration of the same three plates were 19.0 millimeters, the corrected value would be 19.2 millimeters. Plot these corrected values, including the average of the 5.0 micrograms per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L=Corrected zone diameter for the lowest concentration of the standard curve;

H=Corrected zone diameter for the highest concentration of the standard curve;

c=Average zone diameter of 36 readings of the 1.0 microgram per milliliter standard;

a, b, d, e=Corrected average values for the 0.64, 0.80, 1.25, and 1.56 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 1.0 microgram per milliliter solution and three cylinders with the 1.0 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve, at 32° C. to 35° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 1.0 microgram per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 1.0 microgram per milliliter value on the curve. From the standard curve, read the potencies corresponding to these corrected values of zone sizes.

(ii) *Plate assay using Bacillus subtilis.* As an alternative method, proceed as directed in § 141b.101 (a) through (i) of this chapter, except that:

(*a*) In lieu of the directions in § 141b.101(b) of this chapter, prepare the nutrient agar for the base and seed layers as follows:

Beef extract	6.0 gm.
Peptone	10.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.8–8.0 after sterilization.

(*b*) Prepare the standard stock solution as directed in subdivision (i) (*c*) of this subparagraph, and in lieu of the concentrations for the standard curve as prescribed in § 141b.101(d) of this chapter, prepare concentrations of 1.28, 1.60, 2.00, 2.50, and 3.12 micrograms per milliliter from this stock solution and use 2.00 micrograms per milliliter concentration as the reference point.

(*c*) In lieu of the directions in § 141b.101(e) of this chapter, dilute the sample to a final concentration of 2.0 micrograms per milliliter (estimated).

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except administer orally, by means of a cannula or other suitable device, to each of 5 mice, within the weight range of 18 grams to 25 grams, 0.5 milliliter of a solution of the drug in sterile distilled water containing 8.0 milligrams (activity) per milliliter.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a 3.0 percent aqueous solution.

(5) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample into a 25-milliliter volumetric flask. Dissolve in a few milliliters of water, add water to volume, and mix. Transfer the solution to a 200-millimeter tube, determine the angular rotation in a suitable polarimeter, using a sodium light or a 5893 Angstrom filter, and calculate the specific rotation.

(6) *Ash content.* Place approximately 1-gram of the sample, accurately weighed, in a tared, metal-free porcelain crucible, and add 2 milliliters of concentrated H_2SO_4. Completely char on low flame, raising the temperature gradually until fumes of SO_3 disappear. Ignite at a dull-red heat or in a muffle furnace at 1,100° F., cool and weigh.

§ 148o.2 Paromomycin sulfate capsules.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Paromomycin sulfate capsules are composed of paromomycin sulfate, with or without one or more suitable and harmless buffer substances, diluents, lubricants, and binders, enclosed in a suitable and harmless gelatin capsule. Each capsule contains not less than 250 milligrams of paromomycin activity. The moisture content is not more than 5.0 percent. The paromomycin sulfate used conforms to the standards prescribed therefor by § 148o.1(a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The paromomycin sulfate used in making the batch for potency, toxicity, moisture, pH, specific rotation, and ash content.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The paromomycin used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The batch: A minimum of 30 capsules.

(c) In case of an initial request for certification, each other ingredient used: 1 package each consisting of not less than 5 grams.

(4) *Fees.* $0.75 for each capsule in the sample submitted in accordance with subparagraph (3) (ii) (*b*) of this paragraph; $4.00 for each package in the samples submitted in accordance with subparagraph (3) (ii) (*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Proceed as directed in § 148o.1(b) (1), except prepare a stock solution of convenient concentration by pooling the entire contents of a representative number of capsules (usually 5 to 12) in an appropriate-sized volumetric flask and dissolve with 0.1*M* potassium phosphate buffer, pH 8.0. The average paromomycin content is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of activity that each capsule is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148o.3 Paromomycin sulfate syrup.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Paromomycin sulfate syrup is a syrup that contains paromomycin sulfate, with or without one or more suitable and harmless buffer substances, flavorings, colorings, suspending and stabilizing agents, and preservatives, suspended or dissolved in a suitable and harmless vehicle. Each milliliter contains not less than 25 milligrams of paromomycin activity per milliliter. Its pH is not less than 7.5 and not more than 8.5. The paromomycin sulfate used conforms to the requirements of § 148o.1(a) (1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays for:

(*a*) The paromomycin sulfate used in making the batch for potency, toxicity, moisture, pH, specific rotation, and ash content.

(*b*) The batch for potency and pH.

(ii) Samples required:

(*a*) The paromomycin sulfate used in making the batch: 10 packages, each containing approximately equal portions of not less than 300 milligrams.

(*b*) The batch: Minimum of 5 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing not less than 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148o.1 (b)(1), except prepare the sample for assay by removing an appropriate aliquot of the syrup with a suitable syringe and placing in an appropriate volumetric flask. The potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of paromomycin activity that it is represented to contain.

(2) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted sample.

All interested persons are invited to present their views in writing regarding this proposal to the Hearing Clerk, Department of Health, Education, and Welfare, Washington 25, D.C., within 30 days from the date of publication of this notice in the FEDERAL REGISTER. Such views and comments should be filed preferably in quintuplicate.

Proposal for the certification of various other antibiotic drugs will be published in the immediate future.

Dated: April 24, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-4619; Filed, May 1, 1963; 8:45 a.m.]

[21 CFR Parts 146, 148, 148a–148z]

ANTIBIOTIC DRUGS

Proposed Regulations for Tests and Methods of Assay and Certification of Antibiotic Drugs Subject to the 1962 Amendments to Section 507 of the Federal Food, Drug, and Cosmetic Act

The Kefauver-Harris Drug Amendments of 1962 provide that all antibiotic drugs intended for human use shall be subject to the certification provisions of sections 502(1) and 507 of the Federal Food, Drug, and Cosmetic Act. These amendments become effective May 1, 1963. Therefore, the Commissioner of Food and Drugs, pursuant to the provisions of the act (sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357), and under the authority delegated to him by the Secretary of Health, Education, and Welfare (25 F.R. 8625), proposes to issue the following amendments and new regulations to provide for tests and methods of assay and certification of certain antibiotic drugs:

1. It is proposed to amend § 146.1 as follows:

a. By revising the section heading to read:

§ 146.1 Definitions and interpretations applicable to all certifiable antibiotic drugs.

b. By adding to paragraph (a) the following new subparagraphs:

(8) Each of the antibiotic substances produced by the growth of *Streptomyces canus*, and each of the same substances produced by any other means, is a kind of amphomycin.

(9) Each of the antibiotic substances produced by the growth of Streptomyces nodosus, and each of the same substances produced by any other means, is a kind of amphotericin.

(10) Each of the antibiotic substances produced by the growth of Bacillus polymyxa var. garyphalus, and each of the same substances produced by any other means, is a kind of colistin.

(11) Each of the antibiotic substances produced by the growth of Streptomyces orchidaceus, and each of the same substances produced by any other means, is a kind of cycloserine.

(12) Each of the antibiotic substances produced by the growth of Streptomyces erythreus, and each of the same substances produced by any other means, is a kind of erythromycin.

(13) Each of the antibiotic substances produced by the growth of Bacillus brevis, and each of the same substances produced by any other means, is a kind of gramicidin.

(14) Each of the antibiotic substances produced by the growth of Penicillium patulum or Penicillium griseofulvum, and each of the same substances produced by any other means, is a kind of griseofulvin.

(15) Each of the antibiotic substances produced by the growth of Streptomyces kanamyceticus, and each of the same substances produced by any other means, is a kind of kanamycin.

(16) Each of the antibiotic substances produced by the growth of Streptomyces fradiae, and each of the same substances produced by any other means, is a kind of neomycin.

(17) Each of the antibiotic substances produced by the growth of Streptomyces niveus (known also as Streptomyces spheroides), and each of the same substances produced by any other means, is a kind of novobiocin.

(18) Each of the antibiotic substances produced by the growth of Streptomyces noursei, and each of the same substances produce by any other means, is a kind of nystatin.

(19) Each of the antibiotic substances produced by the growth of Streptomyces antibioticus, and each of the same substances produced by any other means, is a kind of oleandomycin.

(20) [Reserved]

(21) Each of the antibiotic substances produced by the growth of Streptomyces rimosus, and each of the same substances produced by any other means, is a kind of oxytetracycline.

(22) Each of the antibiotic substances produced by the growth of Streptomyces rimosus var. paromomycinus, and each of the same substances produced by any other means, is a kind of paromomycin.

(23) Each of the antibiotic substances produced by the growth of Bacillus polymyxa, and each of the same substances produced by any other means, is a kind of polymyxin.

(24) Each of the antibiotic substances produced by the growth of Nocardia lurida, and each of the same substances produced by any other means, is a kind of ristocetin.

(25) Each of the mixtures of antibiotic substances produced by the growth of Bacillus brevis, and each of the same mixtures of substances produced by any other means, is a kind of tyrothricin.

(26) Each of the antibiotic substances produced by the growth of Streptomyces orientalis, and each of the same substances produced by any other means, is a kind of vancomycin.

(27) Each of the antibiotic substances produced by the growth of Streptomyces puniceus or Streptomyces floridae or Actinomyces vinaceus, and each of the same substances produced by any other means, is a kind of biomycin.

c. By adding to paragraph (b) the following new subparagraphs:

(10) The term "amphomycin master standard" means a specific lot of amphomycin designated by the Commissioner as the standard of comparison in determining the potency of the amphomycin working standard.

(11) The term "amphotericin A master standard" means a specific lot of amphotericin A designated by the Commissioner as the standard of comparison in determining the potency of the amphotericin A working standard. The term "amphotericin B master standard" means a specific lot of amphotericin B designated by the Commissioner as the standard of comparison in determining the potency of the amphotericin B working standard.

(12) The term "colistin master standard" means a specific lot of colistin designated by the Commissioner as the standard of comparison in determining the potency of the colistin working standard.

(13) The term "cycloserine master standard" means a specific lot of cycloserine designated by the Commissioner as the standard of comparison in determining the potency of the cycloserine working standard.

(14) The term "erythromycin master standard" means a specific lot of erythromycin designated by the Commissioner as the standard of comparison in determining the potency of the erythromycin working standard.

(15) The term "gramicidin master standard" means a specific lot of gramicidin designated by the Commissioner as the standard of comparison in determining the potency of the gramicidin working standard.

(16) The term "griseofulvin master standard" means a specific lot of griseofulvin designated by the Commissioner as the standard of comparison in determining the potency of the griseofulvin working standard.

(17) The term "kanamycin master standard" means a specific lot of kanamycin designated by the Commissioner as the standard of comparison in determining the potency of the kanamycin working standard.

(18) The term "neomycin master standard" means a specific lot of neomycin designated by the Commissioner as the standard of comparison in determining the potency of the neomycin working standard.

(19) The term "novobiocin master standard" means a specific lot of novobiocin designated by the Commissioner as the standard of comparison in determining the potency of the novobiocin working standard.

(20) The term "nystatin master standard" means a specific lot of nystatin designated by the Commissioner as the standard of comparison in determining the potency of the nystatin working standard.

(21) The term "oleandomycin master standard" means a specific lot of oleandomycin designated by the Commissioner as the standard of comparison in determining the potency of the oleandomycin working standard.

(22) [Reserved]

(23) The term "oxytetracycline master standard" means a specific lot of oxytetracycline designated by the Commissioner as the standard of comparison in determining the potency of the oxytetracycline working standard.

(24) The term "paromomycin master standard" means a specific lot of paromomycin designated by the Commissioner as the standard of comparison in determining the potency of the paromomycin working standard.

(25) The term "polymyxin B master standard" means a specific lot of polymyxin B designated by the Commissioner as the standard of comparison in determining the potency of the polymyxin B working standard.

(26) The term "ristocetin A master standard" means a specific lot of ristocetin A designated by the Commissioner as the standard of comparison in determining the potency of the ristocetin A working standard. The term "ristocetin B master standard" means a specific lot of ristocetin B designated by the Commissioner as the standard of comparison in determining the potency of the ristocetin B working standard.

(27) The term "vancomycin master standard" means a specific lot of vancomycin designated by the Commissioner as the standard of comparison in determining the potency of the vancomycin working standard.

(28) The term "viomycin master standard" means a specific lot of viomycin designated by the Commissioner as the standard of comparison in determining the potency of the viomycin working standard.

2. It is proposed to amend paragraph (c) of § 146.3 *Certification* by changing the phrase "in parts 141a to 141e and § 147.2 of this chapter" to read "issued under this chapter."

3. It is proposed to change the introduction to § 146.10 to read:

§ 146.10 Antibiotics for export.

Drugs containing antibiotic substances subject to certification under section 507 of the act and intended for export will be certified notwithstanding failure to meet the labeling requirements of the applicable monographs if the labeling used for such drugs complies with the following conditions:

* * * * *

4. It is proposed to amend § 146.12 *Antibiotic drugs exempted from certification requirements under section 507 (c) of the Federal Food Drug and Cosmetic Act* in the following respects:

a. By changing the first sentence of paragraph (a) to read:

(a) Section 507(a) of the Federal Food, Drug, and Cosmetic Act directs the Secretary of Health, Education, and Welfare to promulgate regulations to provide for the certification of batches of drugs composed wholly or partly of any kind of antibiotic substance. * * *

b. By changing the item "Antibiotics for fish diseases" in paragraph (b) (3) to read:

Antibiotics for diseases of fish not intended for use as food.

5. It is proposed to amend § 146.22 *Exemptions for manufacturing use* by changing paragraph (a) to read:

(a) Except as provided by paragraphs (c) and (d) of this section, a shipment or other delivery of any certifiable antibiotic drug subject to the regulations in this chapter that is packed in containers of not less than 10,000,000 units of penicillin or 10 grams each of one of the other antibiotic drugs shall be exempt, during the time of introduction into and movement in interstate commerce and the time of holding in the establishment where it is so used, from the requirements of section 502(*l*) of the act, if it conforms to the standards prescribed therefor by the section of the regulations in this chapter which is specifically applicable to such other drug, if the label of each container bears the batch mark of the drug, the number of units or grams per package, and the date on which the latest assay of the drug was completed, and if the person who introduced each shipment or delivery into interstate commerce holds a permit from the Commissioner authorizing shipment for manufacturing use in such establishment.

6. It is proposed to amend § 146.24 as follows:

a. By changing the section heading and the introduction to the section to read:

§ 146.24 Antibiotic powders for diagnostic use.

Dry powders of antibiotic substances, with or without suitable buffer substances and diluents, packaged for dispensing and intended for use solely in laboratory procedures in connection with the diagnosis or treatment of disease and conspicuously so labeled shall be exempt from the certification requirements of sections 502(*l*) and 507 of the act if they comply with all the following conditions:

b. By changing paragraph (a) to read:

(a) The potency and moisture content of the antibiotic used in the manufacture of the powder and the moisture content of the powder comply with the standards prescribed for the antibiotic by the specific regulations issued in this chapter.

7. It is proposed to amend § 146.25 *Antibiotic drugs for use in medicated animal feed* * * * by changing the phrase "regulations in this chapter" in the introduction to the section to read: "regulations in Parts 146a, 146b, 146c, 146d, and 146e of this chapter".

8. It is proposed to amend Title 21 by adding thereto the following new parts:

PART 148—ANTIBIOTIC DRUGS SUBJECT TO CERTIFICATION UNDER THE DRUG AMENDMENTS OF 1962

Sec.
148.1 Definitions and interpretations.
148.2 Packaging requirements.
148.3 Labeling requirements.
148.4 Requests for certification; check tests and assays; samples.
148.5 Fees.

AUTHORITY: §§ 148.1 to 148.5 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148.1 Definitions and interpretations.

The definitions and interpretations of terms found in § 146.1 of this chapter apply also to this Part 148 and to Parts 148a–148z of this chapter.

§ 148.2 Packaging requirements.

Each drug described in Parts 148a to 148z, inclusive, of this chapter shall be packaged in immediate containers complying with the standards that the U.S.P. requires for containers used to package the same dosage form of a drug described in that compendium.

§ 148.3 Labeling requirements.

(a) If it is packaged for dispensing:

(1) It shall be labeled in accordance with the requirements prescribed by § 1.106(b) of this chapter, issued under section 502(f) of the act, unless the regulations pertaining to such drug specifically exempt it from such requirements.

(2) Its labeling shall bear any additional information required for the drug by specific regulations.

(3) Each package shall bear on its outside wrapper or container and the immediate container an expiration date prescribed for the drug by specific regulations, except that the date may be used that is 18, 24, 30, 36, 42, 48, 54, or 60 months after the month during which the batch was certified if the person who requests certification has submitted to the Commissioner results of tests and assays showing that such drug as prepared by him is stable for such period of time. If the manufacturer or repacker of the drug has been exempted from the certification requirements, such date shall be the number of months after the month during which the batch was last assayed and released by the manufacturer or repacker. If an expiration date is used that is longer than the minimum date provided for the drug by specific

regulations, it may be used only if the manufacturer has submitted information to the Commissioner adequate to prove that the drug is stable for such time.

(b) If it is packaged solely for manufacturing use or for repacking, each package shall bear on its outside wrapper or container and the immediate container, the following:

(1) The number of units or micrograms of activity per milligram or per gram, and the number of grams or kilograms in the immediate container.

(2) The batch mark.

(3) The statement "Caution: Federal law prohibits dispensing without prescription."

(4) The statement "For manufacturing use," "For repacking," or "For manufacturing use or repacking," and, if it is not sterile, the statement "nonsterile."

(5) The required expiration date.

§ 148.4 Requests for certification; check tests and assays; samples.

In addition to complying with the requirements of § 146.2 of this chapter, a person who requests certification or check tests and assays of a batch shall submit with his request the following information and samples:

(1) The batch mark of the drug.

(2) The quantity of each ingredient used in making the batch.

(3) The size of the batch, including the number of containers of each size in the batch.

(4) The date of the latest assay of the batch.

(5) The results of tests and assays made by or for him on the batch as required for the drug by specific regulations.

(6) The batch mark(s) of the antibiotic(s) used in making the batch.

(7) Unless previously submitted, the results of tests and assays made by or for him on the antibiotic(s) used in making the batch as required by specific regulations.

(8) The number of accurately representative samples that are required for the batch by specific regulations: In the case of drugs such as dry powders, solutions, ointments, and suspensions, the sample shall be collected by taking single immediate containers, before or after labeling, at such intervals throughout the entire time of packaging the batch that the quantities packaged during the intervals are approximately equal. In no case, however, shall more than 5,000 immediate containers have been packaged during each such interval of sampling, except for a sample collected for sterility testing. In the case of drugs such as tablets or other such unit dosage forms, the sample shall be collected by taking single tablets at such intervals throughout the entire time of tableting the batch that the quantities tableted during the intervals are approximately equal. In no case, however, shall more than 5,000 tablets have been tableted during each interval of sampling, except for a sample collected for time of disintegration. If the person who packages the tablets into dispensing-size containers is not the manufacturer, such sample shall be collected throughout the entire time of packaging the batch into such containers.

(9) Unless previously submitted, the number of accurately representative samples that are required for the antibiotic(s) used in making the batch by the regulations specifically relating to such drug.

(10) In connection with contemplated requests for certification of repacked batches or batches of another drug in the manufacture of which it is to be used, the manufacturer of a batch which is to be so repackaged or used may request the Commissioner to make check tests and assays on a representative sample of such batch. From the information submitted may be omitted results of tests and assays not required for the batch when used in such other drug. The Commissioner shall report to such manufacturer results of such check tests and assays as are so requested.

§ 148.5 Fees.

The fee for the services rendered with respect to each batch certified under the regulations in Parts 148a–148z, inclusive, of this chapter shall accompany the request for certification, or the request for check tests and assays, unless such fee is covered by an advance deposit maintained in accordance with § 146.8(d) of this chapter. Such fee shall be that required for the drug by the specific regulations, plus an additional 30 percent of whatever that fee may be. Also, if the Commissioner considers that investigations other than examination of such samples are necessary to determine whether or not such batch complies with the requirements of § 146.3 of this chapter for the issuance of a certificate, the fee shall include the cost of such investigations.

PART 148a—AMPHOMYCIN

Sec.

148a.1 Amphomycin calcium.

148a.2 Amphomycin calcium-neomycin sulfate cream.

AUTHORITY: §§ 148a.1 to 148a.2 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148a.1 Amphomycin calcium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphomycin calcium is the calcium salt of amphomycin. It is so purified and dried that:

(i) Its potency is not less than 868 micrograms per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 10 percent.

(iv) Its pH in a 2 percent aqueous suspension is not less than 6.0 and not more than 7.5.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, and pH.

(ii) Samples required on the batch: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $4.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described in § 141a.1 of this chapter.

(ii) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(*a*) Make nutrient agar for the base layer as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.0 after sterilization.

(*b*) Make nutrient agar for the seed layer as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.0 after sterilization.

In lieu of preparing the media from the individual ingredients specified in subsections (*a*) and (*b*) of this subdivision, they may be made from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modification of the individual ingredients specified in this subdivision are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(iii) *Preparation of suspension.* The test organism is *Micrococcus flavus* (ATCC 14452). Maintain this organism on slants of nutrient agar prepared as described in subdivision (ii)(b) of this subparagraph and to which has been added 1 milligram of neomycin sulfate per milliliter of agar. Wash the organism from the agar slant with 3 milliliters of sterile sodium chloride solution onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the same type of agar used for the slants. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 32° C. to 35° C., and then wash the resulting growth from the agar surface with 50 milliliters of sterile sodium chloride solution. If an aliquot of this bulk suspension, when diluted 1:40 in the saline solution, gives 25 percent light transmission in a suitable photoelectric colorimeter with a 580-millimicron filter and a test tube with an inside diameter of 12 millimeters as an absorption cell, the bulk suspension is satisfactory for use. It may be necessary to adjust the bulk suspension by dilution, so that an aliquot of the adjusted suspension diluted 1:40 gives 25 percent light transmission. The adjusted bulk suspension, and not the 1:40 dilution of it, is used in preparing the seed layer. Add 0.3 milliliter of the adjusted bulk suspension to each 100

milliliters of seed agar, which has been melted and cooled to 48° C.

(iv) *Preparation of plates.* Add 21 milliliters of the base layer agar prepared as directed in subdivision (ii) (*a*) of this subparagraph to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates and allow it to harden. Use the plates the same day they are prepared. Add 4 milliliters of the inoculum prepared as described in subdivision (iii) of this subparagraph for each plate, tilting back and forth to spread the inoculated agar evenly over the surface. Place six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on 2.8-centimeter radius.

(v) *Working standard.* Dry an appropriate amount of the working standard for 4 hours at 60° C. and a pressure of 5 millimeters of mercury or less. Determine the dry weight and dissolve the dried standard in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a stock standard solution of 1,000 micrograms per milliliter. Store this stock standard solution in a refrigerator, and use for no longer than 14 days.

(vi) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 microgram per milliliter stock standard solution in sufficient 0.1*M* potassium phosphate buffer, pH 8, to obtain concentrations of 4.0, 5.6, 8.0, 11.3, and 16.0 micrograms per milliliter. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution except the 8.0 micrograms per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of three plates fill three cylinders with the 8.0 micrograms per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 8.0-microgram determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 8.0 micrograms per milliliter concentration and the readings of the point tested for each set of three plates and average also all 36 readings of the 8.0 micrograms per milliliter concentration. The average of the 36 readings of the 8.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 8.0 micrograms per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 5.6-microgram concentration the average reading of the 36 readings of the 8.0 micrograms per milliliter concentration is 16.5 millimeters and the average of the 8.0 micrograms per milliliter concentration of this set of three plates is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 5.6 micrograms per milliliter concentration of these same three plates is 15.9 millimeters, the corrected value is then 16.1 millimeters. Plot these corrected values, including the average of the 8.0 micrograms per milliliter concentration, on 2-cycle semi-log paper using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 8.0 micrograms per milliliter standard;

a, b, d, e = Corrected average values for the 4.0, 5.6, 11.3, and 16.0 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(vii) *Preparation of sample.* Dissolve the sample to be tested in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to make a convenient stock solution. Further dilute in buffer to a final concentration of 8.0 micrograms per milliliter (estimated).

(viii) *Plate assay.* Use three plates for each sample. Fill three cylinders on each plate with the 8.0 micrograms per milliliter standard and three cylinders with the 8.0 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 32° C. to 35° C., and measure the diameter of each circle of inhibition. Average the zone readings of the standard and average the zone readings of the sample on the three plates used. If the sample gives a larger average zone size than the average of the standard, add the difference between them to the 8.0-microgram zone size of the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 8.0-microgram value on the curve. From the curve, read the potencies corresponding to these corrected values.

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using as a test dose 0.5 milliliter of a solution containing 1,000 micrograms per milliliter.

(3) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a suspension prepared by adding 20 milligrams per milliliter.

§ 148a.2 Amphomycin calcium-neomycin sulfate cream.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphomycin calcium-neomycin sulfate cream is amphomycin calcium and neomycin sulfate in a suitable and harmless water-soluble cream base. It may contain one or more suitable emulsifiers and preservatives. It may contain cortisone or a suitable derivative of cortisone. Each gram contains not less than 50 milligrams of amphomycin and the equivalent of not less than 3.3 milligrams of neomycin base. The amphomycin calcium used in making the cream conforms to the requirements prescribed by § 148a.1(a)(1). The neomycin sulfate used conforms to the requirements prescribed by § 148i.1(a)(1) of this chapter. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphomycin calcium used in making the batch for potency, moisture, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The batch for amphomycin potency and neomycin sulfate potency.

(ii) Samples required:

(*a*) Amphomycin calcium used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) Neomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch: A minimum of 6 containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*), and (*d*) of this paragraph; $5.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii)(*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Amphomycin content.* Proceed as directed in § 148a.1(b)(1), except in lieu of the directions in § 148.1(b)(1)(vii), prepare the sample as follows: Accurately weigh approximately 2 grams, and place in a blending jar containing 1.0 milliliter of polysorbate 80 and sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a final volume of 200 milliliters. Using a high-speed blender, blend the mixture for 2 minutes and then make the proper estimated dilutions using 0.1*M* potassium phosphate buffer, pH 8.0. Its content of amphomycin is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams per gram that it is represented to contain.

(2) *Neomycin content.* Blend the sample as described in subparagraph (1) of this paragraph and, making the proper estimated dilutions in pH 8.0 buffer, proceed as directed in § 148i.1(b)(1) of this chapter. Its content of neomycin is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams per gram that it is represented to contain.

PART 148b—AMPHOTERICIN

Sec.
148b.1 Amphotericin B.
148b.2 Amphotericin B. intravenous.

AUTHORITY: §§ 148b.1 to 148b.2 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148b.1 Amphotericin B.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Amphotericin B is a yellow to golden-orange powder. It is insoluble in water at pH. 6.0 to 7.0, anhydrous alcohols, esters, ethers, benzene, and toluene. It is soluble in dimethyl formamide and dimethyl sulfoxide. It is so purified and dried that:

(i) Its potency is not less than 750 micrograms per milligram on an anhydrous basis.

(ii) It contains not more than 15 percent of amphotericin A.

(iii) It is sterile.

(iv) It is nontoxic.

(v) It is nonpyrogenic.

(vi) It contains no histamine nor histamine-like substances.

(vii) Its moisture content is not more than 5.0 percent.

(viii) Its pH in a 3 percent aqueous suspension is not less than 5.0 and not more than 8.0.

(ix) It exhibits absorption maxima at 362, 381, and 405 millimicrons when dissolved in dimethyl sulfoxide and diluted with absolute methyl alcohol.

(2) *Labeling.* In addition to the labeling prescribed by § 148.3(b) of this chapter, each package shall bear on its label the statements "Store below 10° C." and "Protect from light and moisture." Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, amphotericin A content, sterility, toxicity, pyrogens, histamine, moisture, pH, and identity.

(ii) Samples required on the batch:

(*a*) For all tests except sterility: 10 packages, each containing not less than 500 milligrams.

(*b*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $10.00 for each package in the sample submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all packages in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Plate assay*—(*a*) *Cylinders (cups).* Use cylinders described in § 141a.1 of this chapter.

(*b*) *Culture media.* Use ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F.

(*1*) Make nutrient agar for preparing the inoculated agar plates as follows:

Peptone	9.4 gm.
Yeast extract	4.7 gm.
Beef extract	2.4 gm.
Sodium chloride	10.0 gm.
Dextrose	10.0 gm.
Agar	23.5 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.0 to 6.2 after sterilization.

(*2*) Make nutrient agar for carrying the organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(*3*) Make nutrient broth for preparing an inoculum of the test organism:

Peptone	10.0 gm.
Dextrose	20.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 5.6 to 5.7 after sterilization.

In lieu of preparing the media from the individual ingredients as specified, they may be prepared from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the specified individual ingredients are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(*c*) *Working standard.* Dry approximately 30 milligrams of the amphotericin B working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight, and dissolve the dried standard in sufficient dimethyl sulfoxide to give a stock solution containing 1,000 micrograms per milliliter. This solution should be used for 1 day only.

(*d*) *Preparation of sample.* Dissolve the sample in sufficient dimethyl sulfoxide to give a convenient stock solution. Further dilute with dimethyl sulfoxide to a concentration of 20 micrograms per milliliter (estimated). Remove an aliquot and dilute to a concentration of 1.0 microgram per milliliter (estimated) using 0.2*M* potassium phosphate buffer, pH 10.5 (dipotassium phosphate, 35 grams; 10*N* sodium hydroxide, 2 milliliters). Make to 1 liter, using distilled water.

(*e*) *Test organism.* The test organism is Saccharomyces cerevisiae (ATCC 9763 or ATCC 2601 no vitamin requirement), which is maintained on slants of agar described in subsection (*b*)(*2*) of this subdivision and transferred once a week. After transfer, the culture is incubated at 37° C. for 24 hours and then kept refrigerated. Prepare the organism suspension by either of the following methods:

(*1*) Inoculate 100 milliliters of the broth medium described under subsection (*b*)(*3*) of this subdivision with a loopful of growth from the agar slant. Incubate the broth for 16 hours to 18 hours at 37° C. This broth culture may be used for several weeks if kept refrigerated.

(*2*) Wash the organism from the agar slant with 3 milliliters of sterile sodium chloride solution onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in subsection (*b*)(*2*) of this subdivision. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 37° C., and then wash the resulting growth from the agar surface with about 30 milliliters of sterile sodium chloride solution. This suspension may be used for several weeks if kept refrigerated. Run test plates to determine the quantity of this broth culture (usually about 2.5 milliliters) or saline suspension (usually about 0.1 milliliter) that should be added to each 100 milliliters of agar to give clear, sharp inhibitory zones of appropriate size.

(*f*) *Preparation of plates.* Melt a sufficient amount of the agar described in subsection (*b*)(*1*) of this subdivision, cool to 48° C., add the proper amount of the test organism as described in subsection (*e*) of this subdivision, and mix thoroughly. Add 8 milliliters of this inoculated agar to each Petri dish (100 millimeters x 22 millimeters, with flat bottom). Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*g*) *Standard curve.* Dilute aliquots of the working stock solution with dimethyl sulfoxide to give concentrations of 40.0, 28.4, 20.0, 14.2, and 10.0 micrograms per milliliter, respectively. Dilute one volume of each of these solutions with 19 volumes of potassium phosphate buffer, pH 10.5, to give final concentrations of 2.0, 1.42, 1.0, 0.71, and 0.5 microgram per milliliter. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution, except the 1.0 microgram per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of the three plates fill three cylinders with the 1.0 microgram per milliliter standard and the other three cylinders with the concentration of the standard concentration under test. Thus, there will be thirty-six 1.0-microgram determinations and nine determinations for each of the other concentrations on the curve. Incubate the plates for 16 hours to 18 hours at 37° C., and measure the diameter of each zone of inhibition. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the concentration tested for each set of three plates, and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.71 microgram per milliliter concentration the average of the 36 readings of the 1.0 microgram per milliliter concentration is 16.0 millimeters and the average of the 1.0 microgram per milliliter concentration of this set of three plates is 15.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 0.71 microgram per milliliter concentration of these same three plates is 15.0 millimeters, the corrected value

is then 15.2 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration on two-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the 1.0 microgram per milliliter standard and three cylinders with the 1.0 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 37° C., and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 1.0 microgram per milliliter zone on the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 1.0 microgram per milliliter value on the curve. From the curves read the potencies corresponding to these corrected values of zone sizes.

(ii) *Turbidimetric assay*—(a) *Culture media.* Use ingredients that conform to the standards, if any, prescribed by the U.S.P. or N.F.

(*1*) Make yeast-beef agar for carrying the organism as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water to make	1,000.0 ml.

pH 6.6 after sterilization.

(2) Make yeast-beef broth for preparing an inoculum of the test organism:

Beef extract	1.5 gm.
Yeast extract	6.5 gm.
Peptone	5.0 gm.
Trypton	10.0 gm.
Dextrose	11.0 gm.
Sodium chloride	3.5 gm.
Dipotassium phosphate	3.68 gm.
Monopotassium phosphate	1.32 gm.
Distilled water to make	1,000.0 ml.

pH 6.6 after sterilization.

(3) Make yeast-beef assay broth:

Beef extract	1.5 gm.
Yeast extract	1.5 gm.
Peptone	5.0 gm.
Dextrose	11.0 gm.
Sodium chloride	3.5 gm.
Dipotassium phosphate	3.68 gm.
Monopotassium phosphate	1.32 gm.
Distilled water to make	1,000.0 ml.

pH 6.6 after sterilization.

In lieu of preparing the media from the individual ingredients as specified, they may be prepared from a dehydrated mixture which, when reconstituted with distilled water, has the same composition as such media. Minor modifications of the specified individual ingredients are permissible if the resulting media possess growth-promoting properties at least equal to the media described.

(*b*) *Working standard and standard solutions.* Use the working standard described in subdivision (i) (*c*) of this subparagraph. Dissolve in dimethyl sulfoxide to obtain a concentration of 2 milligrams per milliliter. Further dilute to obtain a concentration of 30 micrograms per milliliter, using 25 percent dimethyl sulfoxide (one part dimethyl sulfoxide plus three parts distilled water). Use 1 percent potassium phosphate buffer, pH 6.0, and dilute to a concentration of 1 microgram per milliliter. Further dilute this solution with distilled water, and prepare the standard curve containing concentrations of 0.032, 0.04, 0.05, 0.0625, and 0.0781 microgram per milliliter. Add 1.0 milliliter of each concentration to each of six sterile 20 millimeters x 150 millimeters test tubes.

(*c*) *Preparation of sample.* Prepare the sample to be tested, using the same procedure as described for the standard solutions, obtaining six tubes each containing 1.0 milliliter of a solution containing 0.05 microgram per milliliter (estimated).

(*d*) *Preparation of test organism.* The test organism is Candida tropicalis (ATCC 13803), which is maintained on slants of agar described in (*a*) (*1*) of this subdivision. Prepare the daily inoculum by transferring the culture from a stock slant to 100 milliliters of the broth described in (*a*) (*2*) of this subdivision, and incubate for 16 hours to 18 hours at 30° C., with constant shaking. Prepare the daily inoculated assay broth by adding from 3 milliliters to 5 milliliters of a fresh 16 hour to 18 hour broth culture to each liter of assay broth, prepared as directed in (*a*) (*2*) of this subdivision, needed for the test.

(*e*) *Procedure.* To each of the 20 millimeters x 150 millimeters test tubes prepared in (*b*) and (*c*) of this subdivision, add 9.0 milliliters of the inoculated assay broth described in subsection (*d*) of this subdivision, and incubate all standard and sample tubes at 30° C., with constant shaking, for 3 to 5 hours. After incubation, add 0.5 milliliter of a 12 percent formaldehyde solution to each tube, and read the absorbance values in a suitable photoelectric colorimeter, using a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear uninoculated broth prepared as described in (ii) (*a*) (*2*) of this subdivision.

(*f*) *Estimation of potency.* Plot the average values for each concentration of the standard on semilog graph paper with absorbance values on the arithmetic scale and amphotericin concentrations on the logarithmic scale. Construct the best straight line through the points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Absorbance value for the lowest concentration of the standard curve;
H = Absorbance value for the highest concentration of the standard curve;
a, b, c, d, e = Average absorbance values for each concentration of the standard curve.

Plot the values obtained for L and H and connect the points with a straight line. Average the absorbance values for the sample and read the amphotericin concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the amphotericin content of the sample.

(2) *Amphotericin A content*—(i) *Amphotericin A.* Accurately weigh about 20 milligrams of the amphotericin A working standard into a 200-milliliter volumetric flask. Add exactly 20.0 milliliters of dimethyl sulfoxide and dissolve. Make to mark with methyl alcohol, and mix thoroughly. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to mark, and mix thoroughly.

(ii) *Amphotericin B.* Accurately weigh about 50 milligrams of the amphotericin B working standard into a 50-milliliter volumetric flask and proceed as directed in subdivision (i) of this subparagraph.

(iii) *Sample.* Accurately weigh about 50 milligrams of the sample to be tested, and proceed as directed in subdivision (i) of this subparagraph. The standards should be used for 1 day only.

(iv) *Blank.* Pipette 5.0 milliliters of dimethyl sulfoxide into a 50-milliliter volumetric flask. Make to mark with methyl alcohol, and mix. Pipette 4.0 milliliters of this solution into a 50-milliliter volumetric flask. Make to mark with methyl alcohol, and mix thoroughly.

(v) *Procedure.* Use a suitable ultraviolet spectrophotometer and 1-centimeter silica cells. Adjust the instrument to zero with the blank solution. Measure the absorbances of the solutions of standard A, standard B, and the sample at 304 millimicrons and at 282 millimicrons. Calculate the absorptivity of each standard at both wavelengths:

$$\text{Percent amphotericin A}=\frac{[(B\times S_2)-(b\times S_1)]\times 625}{\text{Weight of sample in grams}\times[(B\times a)-(B\times A)]},$$

where:

A = Absorptivity of amphotericin A standard at 282 millimicrons;
B = Absorptivity of amphotericin B standard at 282 millimicrons;
a = Absorptivity of amphotericin A standard at 304 millimicrons;
b = Absorptivity of amphotericin B standard at 304 millimicrons;
S_1 = Absorbance of sample at 282 millimicrons;
S_2 = Absorbance of sample at 304 millimicrons.

(3) *Sterility.* Proceed as directed in § 141a.2 of this chapter using approximately 500 milligrams from each container tested, except that neither penicillinase nor the control tube is used in the test for bacteria.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except administer orally by means of a cannula or other suitable device, to each of 5 mice, 0.4 milliliter of a suspension of the drug containing 50 milligrams per milliliter in 0.5 percent gum acacia solution.

(5) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using as a test dose 0.5 milliliter per kilogram, of an

aqueous solution containing 2 milligrams of activity per milliliter. If no rabbit shows an individual rise in temperature of 1.1° C. or more above its respective control temperature, and if the sum of the three temperature rises does not exceed 3° C., the material under examination meets the requirements for the absence of pyrogen. If one or two rabbits show a temperature rise of 1.1° C. or more, or if the sum of the temperature rises exceeds 3.0° C., repeat the test, using five other rabbits. If not more than three of the eight rabbits show an individual rise in temperature of 1.1° C. or more, and if the sum of the eight temperature rises does not exceed 8.0° C., the material under examination meets the requirements for the absence of pyrogen.

(6) *Histamine.* Using as a test dose 0.6 milliliter per kilogram of a sterile saline solution containing 5.0 milligrams per milliliter, proceed as directed in § 141b.105 of this chapter.

(7) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(8) *pH.* Using a 3.0 percent aqueous suspension, proceed as directed in § 141a.5(b) of this chapter.

(9) *Identity.* Accurately weigh 50.0 milligrams of the sample into a dry 50-milliliter volumetric flask. Add exactly 5 milliliters of dimethyl sulfoxide and dissolve. Make to mark with methyl alcohol, and mix thoroughly. Pipette 4 milliliters of this solution into a 50-milliliter volumetric flask. Add methyl alcohol to the mark, and mix thoroughly. The sample has absorption peaks at identical wavelengths with that of the standard (272, 282, 295, 362, 381, and 405 millimicrons).

§ 148b.2 Amphotericin B intravenous.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Amphotericin B intravenous is a dry powder composed of amphotericin B and sodium desoxycholate and one or more suitable buffering substances. It contains the equivalent of not less than 50 milligrams of amphotericin B. It is sterile. It is nontoxic. It is nonpyrogenic. It contains no histamine nor histamine-like substances. Its moisture content is not more than 8.0 percent. Its pH in an aqueous solution containing 10 milligrams of activity per milliliter is not less than 7.2 and not more than 8.0. The amphotericin B used conforms to the requirements of § 148b.1(a)(1), except that its amphotericin A content is not more than 5 percent. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* In addition to the labeling requirements prescribed by § 148.3 of this chapter, each package shall bear on its label or labeling the following statement: "For intravenous infusion in hospitals only." Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The amphotericin B used in making the batch for potency, amphotericin A content, and identity.

(*b*) The batch for potency, sterility, toxicity, pyrogens, histamine, moisture, and pH.

(ii) Samples required:

(*a*) Amphotericin B used in making the batch: 10 packages, each containing approximately equal portions of not less than 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: A minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*3*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii)(*a*) and (*b*) (*1*) and (*3*) of this paragraph; $10.00 for all immediate containers in the samples submitted in accordance with subparagraph (3)(ii)(*b*)(2) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency.* Dilute the sample in sufficient dimethyl sulfoxide to give a convenient stock solution, and proceed as directed in §148b.1(b)(1). Its potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Sterility.* Proceed as directed in §141a.2 of this chapter, using the entire contents of each container tested, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.3 milliliter of an aqueous solution containing 50 micrograms of activity per milliliter.

(4) *Pyrogens.* Proceed as directed in § 148b.1(b)(5).

(5) *Histamine.* Proceed as directed in § 148b.1(b)(6).

(6) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using an aqueous solution containing 10 milligrams of activity per milliliter.

PART 148c—COLISTIN SULFATE

Sec.

148c.1 Colistin sulfate.
148c.2 Colistin sulfate-neomycin sulfate otic solution.
148c.3 Colistin sulfate for oral suspension.
148c.4 Colistin methane sulfonate sodium.

AUTHORITY: §§ 148c.1 to 148c.4 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148c.1 Colistin sulfate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate is the white to slightly yellow, odorless sulfate salt of a kind of colistin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 500 micrograms per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 7.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 4.0 and not more than 7.0.

(v) It gives a positive identity test for colistin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b). Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4, of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, pH, and identity.

(ii) Samples required on the batch: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $4.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(ii) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(*a*) Nutrient agar for the base layer and for preparing the inoculated agar plates as follows:

	Base layer	*Seed layer*
Pancreatic digest of casein	17.0 gm.	17.0 gm.
Papaic digest of soybean	3.0 gm.	3.0 gm.
Sodium chloride	5.0 gm.	5.0 gm.
Dextrose	2.5 gm.	2.5 gm.
Dibasic potassium phosphate	2.5 gm.	2.5 gm.
Polysorbate 80	------	10.0 ml.
Agar	20.0 gm.	12.0 gm.
Distilled water to make	1,000.0 ml.	1,000.0 ml.
Final pH (after sterilization)	7.2–7.3	7.2–7.3

(*b*) Make nutrient agar for carrying the organism as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Glucose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s	1,000.0 ml.

pH 6.5–6.6 after sterilization.

In lieu of preparing the medium from the individual ingredients specified, it can be made from a dehydrated mixture that, when reconstituted with distilled water, has the same composition as such medium. Minor modifications of the specified individual ingredients are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(iii) *Preparation of test organism.* The test organism used is Bordetella bronchiseptica (ATCC 4617), and it is maintained on the nutrient agar described in subdivision (ii)(*a*) of this subparagraph. Inoculate a Roux bottle containing 300 milliliters of this agar from a stock slant and incubate 24 hours at 32° C.–35° C. Wash the resulting growth with 50 milliliters of sterile sodium chloride solution and standard-

ize the organism suspension so that a 1:20 dilution in saline will give 25 percent light transmission, using a filter having a wavelength of 580 millimicrons. Use 0.3 milliliter of this standardized bacterial suspension (not the 1:20 dilution) to inoculate each 100 milliliters of melted seed layer agar (cooled to 48° C.–50° C.).

(iv) *Preparation of plates.* Use plates the same day they are prepared. Evenly distribute 21.0 milliliters of the melted base layer agar on each Petri dish (20 millimeters x 100 millimeters); cover with porcelain tops, glazed on the outside, and allow the agar to harden. Evenly spread 4.0 milliliters of the inoculated seed layer agar over the surface of the plate. After the seed layer has hardened, placed six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(v) *Working standard.* Dry an appropriate amount of the working standard for 3 hours at 60°C. and a pressure of 5 millimeters or less. Determine the dry weight and prepare a stock solution of 1,000 micrograms per milliliter by diluting this dried working standard with sterile 10 percent potassium phosphate buffer, pH 6.0.

(vi) *Standard curve.* Prepare concentrations for the standard curve by diluting the working standard stock solution (1,000 micrograms per milliliter) with sterile 10 percent potassium phosphate buffer, pH 6.0, to give final concentrations of 0.64, 0.80, 1.0, 1.25, and 1.56 micrograms per milliliter. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution, except the 1.0 microgram per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of the three plates fill three cylinders with the 1.0 microgram per milliliter standard and the other three cylinders with the concentration of the standard concentration under test. Thus, there will be 36 one-microgram determinations and nine determinations for each of the other concentrations on the curve. Incubate the plates for 16 hours to 18 hours at 37° C. and measure the diameter of each zone of inhibition. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the concentration tested for each set of three plates, and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 1.25 microgram per milliliter concentration the average of the 36 readings of the 1.0 microgram per milliliter concentration is 18.0 milliliters, and the average of the 1.0 microgram per milliliter concentration of this set of three plates is 17.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 1.25 microgram per milliliter concentration of those same three plates is 19.6 millimeters, the corrected value is then 19.8 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on two-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points.

(vii) *Preparation of sample.* Dissolve an accurately weighed quantity of the sample in sufficient 10 percent potassium phosphate buffer, pH 6.0, to give a convenient stock solution. Further dilute in the same buffer to obtain an estimated concentration of 1.0 microgram per milliliter.

(viii) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the 1.0 microgram per milliliter standard and three cylinders with the 1.0 microgram per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 37° C. and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 1.0 microgram per milliliter zone on the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 1.0 microgram per milliliter value on the curve. From the curve, read the potencies corresponding to these corrected values of zone sizes.

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.5 milliliter of a sterile distilled water solution containing 600 micrograms of colistin sulfate activity per milliliter.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter, using a sample of about 300 milligrams.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Identity.* Weigh about 20 milligrams of sample, add 2 milliliters of 2.5 percent sodium acetate solution and 0.2 milliliter of 0.5 percent triketohydrindene hydrate solution, and bring to boil. A deep-purple color results.

§ 148c.2 Colistin sulfate-neomycin sulfate otic solution with hydrocortisone.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate-neomycin sulfate otic solution with hydrocortisone is colistin sulfate, neomycin sulfate, hydrocortisone, and one or more buffer substances and dispersing agents, dissolved in a suitable and harmless vehicle. Each milliliter contains not less than 3.0 milligrams of colistin base activity (as the sulfate) and not less than 3.3 milligrams of neomycin base activity (as the sulfate). Its pH is not less than 4.8 and not more than 5.2. The colistin sulfate used conforms to the standards prescribed therefor by § 148c.1(a)(1), except for toxicity. The neomycin sulfate used conforms to the standards prescribed by § 148i.1(a)(1) of this chapter, except for sterility, pyrogens, toxicity and histamine. Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The colistin sulfate used in making the batch for potency, moisture, pH, and identity.

(*b*) The neomycin sulfate used in making the batch for potency, moisture, pH, and identity.

(*c*) The batch for colistin potency, neomycin potency, and pH.

(ii) Samples required:

(*a*) The colistin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The neomycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*c*) The batch: A minimum of seven immediate containers.

(*d*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each immediate container in the samples submitted in accordance with subparagraph (3)(ii)(*a*), (*b*), and (*d*) of this paragraph; $5.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii)(*c*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Colistin content.* Proceed as directed in § 148c.1(b)(1), except prepare the sample as follows: Thoroughly mix the sample and transfer an accurately measured aliquot of the sample into a 100-milliliter volumetric flask. Fill the flask to mark with 10 percent potassium phosphate buffer, pH 6.0. Further dilute to the proper estimated concentration in 10 percent potassium phosphate buffer, pH 6.0. The average potency is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams per milliliter that it is represented to contain.

(2) *Neomycin content.* Proceed as directed in § 148i.1(b)(1) of this chapter, except prepare the sample as follows: Thoroughly mix the sample and transfer an accurately measured aliquot to a 100-milliliter volumetric flask. Fill to mark with 0.1*M* potassium phosphate buffer, pH 8.0. Further dilute to the proper estimated concentration in pH 8.0 buffer. The average potency is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams per milliliter that it is represented to contain.

(3) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using the undiluted solution or suspension.

§ 148c.3 Colistin sulfate for oral suspension.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin sulfate for oral suspension is a dry mixture of colistin sulfate, with or without one or more suitable and harmless buffer substances, suspending and dispersing agents, diluents, colorings, and flavorings. The colistin sulfate content is not less than 5.0 milligrams per milliliter of the reconstituted suspension. The moisture content is not more than 3.0 percent. The colistin sulfate used conforms to the standards prescribed by § 148c.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled as prescribed in § 148.3, of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirments of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The colistin sulfate used in making the batch for potency, toxicity, pH, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) The colistin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: A minimum of 6 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing not less than approximately 5 grams.

(4) *Fees.* $4.00 for each container submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148c.1(b)(1), except prepare the sample as follows: Reconstitute the suspension as directed in the label. Dissolve and further dilute 5 milliliters of the reconstituted suspension in 1.0 percent potassium phosphate buffer, pH 6.0.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148c.4 Colistin methane sulfonate sodium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Colistin methane sulfonate sodium is a white to slightly yellowish-brown methane sulfonate sodium salt of a kind of colistin, or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 300 micrograms of colistin base per milligram.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine or histamine-like substances.

(vi) Its moisture content is not more than 9.0 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 7.0 and not more than 9.5.

(viii) It gives a positive identity test for colistin methane sulfonate.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, histamine, moisture, pH, and identity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 containers, each containing not less than 500 milligrams.

(*b*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $10.00 for each container in the sample submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148c.1(b)(1) of this chapter.

(2) *Sterility.* Use the entire contents of a container or the equivalent of approximately 500 milligrams of colistin sulfate activity from each large container and proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using a test dose of 1.0 milliliter per kilogram of a solution in sterile, pyrogen-free distilled water, containing 10 milligrams of colistin sulfate activity per milliliter.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.5 milliliter of a solution in sterile distilled water containing 1.5 milligrams of colistin sulfate activity per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using a test dose of 1 milliliter per kilogram of a solution containing 3.0 milligrams of colistin base per milliliter.

(6) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter, using a sample of not less than 100 milligrams.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a solution of 10 milligrams per milliliter.

(8) *Identity.* To about 20 milligrams of sample add 2 milliliters of 2.5 percent sodium acetate solution and 0.2 milliliter of 0.5 percent triketohydrindene hydrate solution. Bring to boil. No pink color results. To about 20 milligrams of sample add 2 milliliters of dilute hydrochloric acid. Reflux for about 2 minutes, neutralize with 10 percent sodium hydroxide, and add sodium acetate. A deep-purple color results.

PART 148d—CYCLOSERINE

Sec.

148d.1 Cycloserine.
148d.2 Cycloserine capsules.
148d.3 Cycloserine capsules with isoniazid.

AUTHORITY: §§ 148d.1 to 148d.3 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148d.1 Cycloserine.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cycloserine is a white to slightly yellowish crystalline compound. It has the chemical structure D-4-amino-3-isoxazolidone. It is so purified that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 1.0 percent.

(iv) Its pH in a 10 percent aqueous solution is not less than 5.5 and not more than 6.5.

(v) Its heavy metals content is not more than 30 parts per million.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency (chemical and biological), toxicity, moisture, pH, crystallinity, and heavy metals.

(ii) Samples of the batch: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Colorimetric assay*—(*a*) *Stock standard solution.* Dry approximately 100 milligrams of the working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and dissolve the dried working standard in sufficient 0.1*N* sodium hydroxide to give a solution containing 1,000 micrograms per milliliter.

(*b*) *Standard curve solutions.* Pipette accurately 0.0, 1.0, 5.0, 10.0, 15.0, and 20.0 milliliters of the stock standard solution to each of six 100-milliliter volumetric flasks, dilute to 100 milliliters with 0.1*N* sodium hydroxide and mix thoroughly.

(*c*) Reagents:

(*1*) Acetic acid—1.0*N* solution.

(*2*) Sodium hydroxide—4.0*N* and 0.1*N* solutions.

(*3*) Sodium nitroprusside—4.0 percent solution: Dissolve 4.0 grams in sufficient distilled water to make 100.0 milliliters. Mix well. Store in amber bottle.

(*4*) Oxidized nitroprusside reagent—Mix equal parts of 4 percent sodium nitroprusside solution and 4.0*N* sodium hydroxide, and let stand for 1 hour before using. Prepare daily, and store in an amber bottle.

(*d*) *Procedure.* Transfer approximately 100 milligrams of sample, accurately weighed, to a 100-milliliter volumetric flask. Dissolve in sufficient 0.1*N* sodium hydroxide to measure exactly 100 milliliters. Mix thoroughly and transfer 10 milliliters to a second 100-milliliter volumetric flask, and mix thoroughly. Transfer exactly 1.0 milliliter of each of the standard curve solutions and of the sample solution to re-

spective test tubes. Add exactly 3.0 milliliters of 1.0*N* acetic acid to each of the test tubes. Mix thoroughly. Add exactly 1.0 milliliter of oxidized nitroprusside reagent to each test tube and mix thoroughly. Allow the tubes to stand at room temperature for at least 10 minutes in order that maximum color intensity may develop. Using the solution containing 0.0 milliliter of working standard as a blank, determine the absorbances of the solutions at 625 millimicrons in a suitable spectrophotometer. Plot concentration versus absorbance on linear graph paper. The curve may deviate slightly from a straight line. The standard curve solutions equal 0, 10, 50, 100, 150, and 200 micrograms of cycloserine, respectively.

(*e*) Calculations:

$$\text{Micrograms cycloserine per milligram} = \frac{\text{Concentration in micrograms from calibration curve} \times 1{,}000}{\text{Weight of original sample in milligrams}}$$

(ii) *Plate assay*—(*a*) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(*b*) *Culture media.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F.

(*1*) Base layer agar:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

(*2*) Seed layer agar (also used for maintaining test organism):

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Dextrose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 6.5 to 6.6 after sterilization.

In lieu of preparing the medium from the individual ingredients specified, it can be made from a dehydrated mixture that, when reconstituted with distilled water, has the same composition as such medium.

(*c*) *Preparation of test organism.* The test organism used is Staphylococcus aureus (ATCC 6538). Incubate a seed agar slant which has been streaked with the organism, for 24 hours at 32° C.–35° C. Wash the slant with 3 milliliters of sterile sodium chloride solution with the aid of glass beads onto a Roux bottle containing 300 milliliters of seed agar. Incubate for 24 hours at 32° C.–35° C., and wash with 50 milliliters of sterile sodium chloride solution into an Erlenmeyer flask. Using a suitable photoelectric colorimeter with a filter having a wavelength of 580 millimicrons, adjust the suspension so that a 1:20 dilution with saline will give 25 percent light transmission. Determine by test plates the amount of inoculum to be added to the seed layer to obtain satisfactory zones (usually 2 percent of a 1:50 dilution).

(*d*) *Preparation of plates.* Use plates the same day they are prepared. Evenly distribute 10.0 milliliters of the melted base layer agar on each Petri dish (20 millimeters x 100 millimeters); cover with porcelain tops, glazed on the outside, and allow the agar to harden. Evenly spread 4.0 milliliters of the inoculated seed layer agar over the surface of the plate. After the seed layer has hardened, place six cylinders on the inoculated agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*e*) *Standard.* Dry an appropriate amount of the working standard for 3 hours at 60° C. with a pressure of 5 millimeters or less. Determine the dry weight and dissolve the dried working standard in sufficient distilled water to obtain a stock solution of 1,000 micrograms per milliliter.

(*f*) *Standard curve.* Prepare concentrations for the standard curve by diluting the working standard stock solution (1,000 micrograms per milliliter) in 1 percent potassium phosphate buffer, pH 6.0, to give final concentrations of 32, 40, 50, 62.5, and 78.1 micrograms per milliliter. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution, except the 50 micrograms per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of the three plates fill three cylinders with 50 micrograms per milliliter standard and the other three cylinders with the concentration of the standard concentration under test. Thus, there will be thirty-six 50-microgram determinations and nine determinations for each of the other concentrations on the curve. Incubate the plates for 16 hours to 18 hours at 30° C., and measure the diameter of each zone of inhibition. Average the readings of the 50 micrograms per milliliter concentration and the readings of the concentration tested for each set of three plates, and average also all 36 readings of the 50 micrograms per milliliter concentration. The average of the 36 readings of the 50 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 50 micrograms per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 62.5 micrograms per milliliter concentration the average of the 36 readings of the 50 micrograms per milliliter concentration is 18.0 millimeters and the average of the 50 micrograms per milliliter concentration of this set of three plates is 17.8 millimeters, the correction is +0.2 millimeter. If the average reading of the 62.5 micrograms per milliliter concentration of those same three plates is 19.6 millimeters, the corrected value is then 19.8 millimeters. Plot these corrected values, including the average of the 50 micrograms per milliliter concentration, on two-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points.

(*g*) *Preparation of sample.* Dissolve an accurately weighed quantity of the sample in 1 percent potassium phosphate buffer, pH 6.0, to an estimated concentration of 50 micrograms per milliliter.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the 50 micrograms per milliliter standard and three cylinders with the 50 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate the plates for 16 hours to 18 hours at 30° C. and then measure the diameter of each zone of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 50 micrograms per milliliter zone on the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 50 micrograms per milliliter value on the curve. From the curves, read the potencies corresponding to these corrected values of zone sizes.

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using a test dose of 0.5 milliliter of a solution in sterile distilled water containing 32 milligrams per milliliter.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(4) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a solution with a concentration of 100 milligrams per milliliter.

(5) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(6) *Heavy metals*—(i) *Reagents*—(*a*) Nitric acid.

(*b*) Sulfuric acid.

(*c*) Hydrochloric acid.

(*d*) Ammonia test solution: Each 100 milliliters of solution contains 9.5 percent to 10.5 percent ammonia.

(*e*) 10 percent acetic acid.

(*f*) Saturated hydrogen sulfide solution, made by passing hydrogen sulfide into cold water.

(*g*) Lead nitrate stock solution: Dissolve 159.8 milligrams of lead nitrate in 10 milliliters of water to which has been added 1.0 milliliter of nitric acid, then dilute with water to 1,000 milliliters. Prepare and store this solution in glass containers free from soluble lead salts.

(*h*) Standard lead solution: Dilute 10 milliliters of lead nitrate stock solution, accurately measured, with water to 100 milliliters. This solution must be freshly prepared. Each milliliter of standard lead solution contains the equivalent of 0.01 milligram of lead. When 0.1 milliliter of standard lead solution is employed to prepare the standard to be compared with a solution of 1.0 gram of the substance being tested, the comparison solution thus prepared contains the equivalent of 1 part of lead per million parts of the substance tested.

(ii) *Procedure.* Transfer 1.0 gram of sample to a porcelain crucible, carefully ignite at a low temperature until thoroughly charred, add 2 milliliters of nitric

acid, 5 drops of sulfuric acid, and cautiously heat until white fumes are evolved. Then ignite in a muffle furnace at 500° C. to 600° C. until the carbon is consumed. Cool, add 2 milliliters of hydrochloric acid, and slowly evaporate on a steam bath to dryness. Moisten the residue with 1 drop of hydrochlorid acid, add 10 milliliters of hot water, and digest for 2 minutes. Add, dropwise, ammonia test solution until the solution is just alkaline to litmus paper; then add dropwise, 10 percent acetic acid until the solution is slightly acid to litmus, and then add 2 milliliters in excess. Filter, if necessary; wash the crucible and filter with about 10 milliliters of water and dilute to 25 milliliters. Transfer to a Nessler tube and add 10 milliliters of saturated hydrogen sulfide solution. The color is no darker than that of a control containing, in the same volume, 3.0 milliliters of standard lead solution, 2 milliliters of 10 percent acetic acid, and 10 milliliters of saturated hydrogen sulfide solution.

§ 148d.2 Cycloserine capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cycloserine capsules are capsules composed of crystalline cycloserine, with or without one or more suitable and harmless buffer substances, diluents, binders, and lubricants. Each capsule contains not less than 250 milligrams of cycloserine. The moisture content is not more than 1.0 percent. The cycloserine used conforms to the requirements prescribed by § 148d.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* In addition to the labeling prescribed by § 148.3 of this chapter, the labeling of each package shall bear a warning to the effect that the drug is to be used in patients with tuberculosis who fail to respond to treatment with isoniazid, streptomycin, para-aminosalicylic acid, viomycin, pyrazinamide, or combinations of these drugs, and that the drug may cause serious reactions such as convulsive seizures and mental disturbances. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Cycloserine used in making the batch for potency (chemical and biological), toxicity, pH, crystallinity, and heavy metals.

(*b*) The batch for potency (chemical and biological), and moisture.

(ii) Samples required:

(*a*) Cycloserine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 30 capsules.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each, containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the samples submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph; $1.00 for each capsule in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Chemical colorimetric assay.* (*a*) Reagents:

(*1*) Acetic acid—1.0*N* solution.

(*2*) Sodium hydroxide—4.0*N* and 0.1*N* solutions.

(*3*) Sodium nitroprusside—4.0 percent solution: Dissolve 4.0 grams in sufficient distilled water to make 100.0 milliliters. Mix well. Store in amber bottle.

(*4*) Oxidized nitroprusside reagent—Mix equal parts of the 4.0 percent sodium nitroprusside solution and 4.0*N* sodium hydroxide, and let stand for 1 hour before using. Prepare daily, and store in amber bottle.

(*5*) Cycloserine standard solution—Prepare a standard solution of the cycloserine working standard in 0.1*N* sodium hydroxide to contain 100 micrograms per milliliter.

(*b*) *Procedure.* Transfer the contents of 10 capsules into a 1,000 milliliter volumetric flask. Add 0.1*N* sodium hydroxide to dissolve the sample, and add sufficient 0.1*N* sodium hydroxide to measure 1,000 milliliters. Mix well, and filter. Dilute an aliquot of the filtrate with sufficient 0.1*N* sodium hydroxide to give a concentration of 0.1 milligram per milliliter (estimated) and mix well. Pipette exactly 1.0 milliliter of the working standard solution and 1.0 milliliter of the sample solution into separate test tubes. Add exactly 3.0 milliliters of 1.0*N* acetic acid and exactly 1.0 milliliter of oxidized nitroprusside reagent to each of the test tubes; then mix thoroughly. Allow the tubes to stand at room temperature for 10 minutes–15 minutes, in order that maximum color intensity may develop. Using a reagent blank, determine the absorbance of the solutions at 625 millimicrons in a suitable spectrophotometer.

Calculation:

$$\text{Milligrams of cycloserine per capsule} = \frac{\text{Sample absorbance}}{\text{Standard absorbance}} \times \text{Labeled potency per capsule in milligrams.}$$

(ii) *Biological assay.* Using a high-speed blender, prepare an appropriate number of capsules in 500 milliliters of 1 percent potassium phosphate buffer, pH 6.0, and proceed as directed in § 148d.1(b)(1)(ii). The average potency is satisfactory if it contains not less than 90 percent and not more than 115 percent of the number of milligrams of cycloserine that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

§ 148d.3 Cycloserine capsules with isoniazid.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Cycloserine capsules with isoniazid are capsules composed of crystalline cycloserine and isoniazid, with or without, one or more suitable and harmless buffer substances, diluents, binders, and lubricants. Each capsule contains not less than 250 milligrams of cycloserine and not less than 150 milligrams of isoniazid. The moisture content is not more than 1.0 percent. The cycloserine used conforms to the requirements prescribed by § 148d.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* In addition to the labeling prescribed by § 148.3 of this chapter, the labeling of each package shall bear a warning to the effect that the drug is to be used in patients with tuberculosis who fail to respond to treatment with isoniazid, streptomycin, para-aminosalicyclic acid, viomycin, pyrazinamide, or combinations of these drugs and that the drug may cause serious reactions such as convulsive seizures and mental disturbances. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.3 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Cycloserine used in making the batch for potency (chemical and biological), toxicity, pH, crystallinity, and heavy metals.

(*b*) The batch for cycloserine content (chemical and biological), isoniazid content, and moisture.

(ii) Samples required:

(*a*) Cycloserine used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 30 capsules.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each package in the sample submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph; $1.50 for each capsule in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Chemical assays for cycloserine and isoniazid*—(*a*) *Preparation of sample.* Transfer the entire contents of four representative capsules to a 1,000-milliliter volumetric flask. Dilute to volume with distilled water, and mix well. Filter about 30 milliliters of the solution, discarding the first few milliliters of filtrate. Collect the remaining filtrate. Transfer a 10.0-milliliter aliquot of the filtrate to a 1,000-milliliter volumetric flask, dilute to volume with 0.1*N* hydrochloric acid, and mix well.

(*b*) *Preparation of cycloserine standard.* Accurately weigh about 30 milligrams of the cycloserine working standard and dissolve in sufficient distilled water to give a concentration of 1.0 milligram per milliliter. Transfer a 10.0-milliliter aliquot of this solution to a 1,000-milliliter volumetric flask. Dilute to volume with 0.1*N* hydrochloric acid, and mix well.

(*c*) *Preparation of isoniazid standard.* Accurately weigh about 30 milligrams of

pure isoniazid and dissolve in sufficient distilled water to give a concentration of 1.0 milligram per milliliter. Transfer a 10.0-milliliter aliquot of this solution to a 1,000-milliliter volumetric flask. Dilute to volume with 0.1*N* hydrochloric acid, and mix well.

(*d*) *Procedure.* Adjust a suitable ultraviolet spectrophotometer to 100 percent light transmission with 0.1*N* hydrochloric acid in the absorption cell. Determine the absorbance values for the sample, the cycloserine standard, and the isoniazid standard at the absorption peaks at 219 millimicrons and 268 millimicrons. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 210 millimicrons to 290 millimicrons. If a nonrecording spectrophotometer is used, determine the absorbance values at the absorption peaks. (The exact position of the peaks should be determined for the particular instrument used.)

(*e*) *Calculations*—(*1*) Cycloserine content: Determine the ratio (*R*) of the absorbance of the isoniazid standard at 219 millimicrons to its absorbance at 268 millimicrons.

$$R = \frac{\text{Absorbance of isoniazid standard at 219 millimicrons}}{\text{Absorbance of isoniazid standard at 268 millimicrons}}.$$

Correct the absorbance value of the sample at 219 millimicrons for the isoniazid equivalent as follows:

$$A_c = (A_1 - A_2 \times R),$$

where:

A_c = Corrected absorbance at 219 millimicrons;
A_1 = Absorbance of sample at 219 millimicrons;
A_2 = Absorbance of sample at 268 millimicrons.

Calculate the cycloserine content as follows:

$$\text{Milligrams of cycloserine per capsule} = \frac{A_c}{A_s} \times 250,$$

where:

A_s = Absorbance of cycloserine standard at 219 millimicrons.

The content of cycloserine is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams of cycloserine that it is represented to contain.

(*2*) *Isoniazid content*—(*i*) *Preparation of sample.* Use the solution prepared as directed in subdivision (i) (*a*) of this subparagraph.

(*ii*) *Preparation of standard.* Use the solution prepared as directed in subdivision (i) (*c*) of this subparagraph.

(*iii*) *Procedure.* Proceed as directed in subdivision (i) (*d*) of this subparagraph and obtain the absorbance of the sample and the isoniazid standard at the absorption peak at 268 millimicrons. Calculate as follows:

$$\text{Milligrams of isoniazid per capsule} = \frac{A_2 \times 250}{A_1},$$

where:

A_1 = Absorbance of isoniazid standard at 268 millimicrons;
A_2 = Absorbance of sample at 268 millimicrons.

The content of isoniazid is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams that it is represented to contain.

(ii) *Plate assay method for cycloserine content.* Proceed as directed in § 148d.-2(b)(1)(ii). The average content of cycloserine in each capsule is satisfactory if it contains not less than 85 percent and not more than 120 percent of the number of milligrams that each capsule is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

PART 148f—GRAMICIDIN

§ 148f.1 Gramicidin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Gramicidin is the white, or nearly white, odorless, crystalline compound of a kind of gramicidin or a mixture of two or more such compounds. It is so purified and dried that:

(i) It has a potency of not less than 900 micrograms of activity per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 3 percent.

(iv) The difference between the absorptivity value at the maximum occurring at 282 millimicrons and the absorptivity value at the minimum occurring at 247 millimicrons is 100±4 percent of the difference obtained with the gramicidin working standard.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, crystallinity, and identity.

(ii) Samples required of the batch: Ten packages, each containing approximately 500 milligrams.

(4) *Fees.* $4.00 for each immediate container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Culture medium.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. Make nutrient broth for preparing an inoculum of the test organism, as follows:

Peptone	5.0 gm.
Yeast extract	1.5 gm.
Beef extract	1.5 gm.
Sodium chloride	3.5 gm.
Dextrose	1.0 gm.
Dipotassium phosphate	3.68 gm.
Potassium dihydrogen phosphate	1.32 gm.
Distilled water, q.s.	1,000.00 ml.

pH 7.0 after sterilization.

In lieu of preparing the medium from the individual ingredients specified above, the nutrient broth may be made from a dehydrated mixture that, when reconstituted with distilled water, has the same composition as such medium. Minor modifications of the individual ingredients specified are permissible if the resulting medium possesses growth-promoting properties at least equal to the medium described.

(ii) *Working standard.* Dry an appropriate amount of the gramicidin working standard for 3 hours at 60° C. and a pressure of 5 millimeters or less. Determine the dry weight and dissolve in sufficient 95 percent alcohol to obtain stock solution of 1,000 micrograms per milliliter. Keep this stock solution in the refrigerator. Do not use it later than 30 days after it is made.

(iii) *Preparation of sample.* Dissolve an appropriate quantity of the sample in a volume of 95 percent ethyl alcohol to obtain a convenient stock solution. Make further dilutions volumetrically in 95 percent ethyl alcohol to obtain a final concentration of 0.04 microgram per milliliter (estimated).

(iv) *Preparation of test organism.* The test organism is Streptococcus faecalis (ATCC 14506) which is maintained in 100-milliliter quantities of the nutrient broth described in subdivision (i) of this subparagraph. For the test, prepare a fresh subculture by transferring a loopful of the stock culture to 100 milliliters of nutrient broth, and incubate 16 hours to 18 hours at 37° C. For the daily inoculum, use 6.0 milliliters of the subculture for each 1,000 milliliters of nutrient broth needed in the assay.

(v) *Procedure.* Prepare solutions for the daily standard curve by diluting an aliquot of the stock working standard solution prepared as described in subdivision (ii) of this subparagraph in 95 percent ethyl alcohol to obtain concentrations of 0.028, 0.034, 0.040, 0.048, and 0.058 microgram per milliliter. Place 0.1 milliliter of each concentration of the standard curve and of the sample solution prepared as described in subdivision (iii) of this subparagraph in each of three replicate test tubes, 16 millimeters x 125 millimeters (outside dimensions). To each tube, add 9 milliliters of the inoculated broth described in subdivision (iv) of this subparagraph and place immediately in a water bath at 37° C. for 3 hours to 4 hours. Remove the tubes and add 0.5 milliliter of a 12 percent solution of formaldehyde to each tube. Read the absorbance values of each tube in a suitable photoelectric colorimeter, using a wavelength of 530 millimicrons. Set the instrument at zero absorbance with clear, uninoculated broth prepared as described in subdivision (i) of this subparagraph.

(vi) *Estimation of potency.* Plot the average absorbance values for each concentration of the standard on two-cycle semi-logarithmic graph paper with absorbance values on the arithmetic scale and concentrations on the logarithmic scale. Construct the best straight line through the points either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3c+2d+e-a}{5},$$

where:

L = Absorbance value for the lowest concentration of the curve;
H = Absorbance value for the highest concentration of the standard curve;
a, b, c, d, e = Average absorbance values for each concentration of the standard curve.

Plot the values obtained for L and H and connect the points with a straight line. Average the absorbance value for the sample and read the gramicidin concentration from the standard curve. Multiply the concentration by appropriate dilution factors to obtain the gramicidin content of the sample.

(2) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except inject subcutaneously with 0.5 milliliter of a suspension of the sample prepared by adding 2 milligrams activity (estimated) of gramicidin mixed well in 4 milliliters of sterile 1 percent aqueous solution of acacia.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(4) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(5) *Identity.* Prepare 0.0075 percent solutions in ethyl alcohol of the sample and of the gramicidin working standard. Using a suitable recording spectrophotometer with 1-centimeter cells, record the ultraviolet absorbance spectrum of each solution from 220 millimicrons to 320 millimicrons. The ultraviolet absorbance spectrum of the sample solution should compare qualitatively to that of the working standard solution. Determine the absorbance at the maximum occurring at 282 millimicrons and at the minimum occurring at 247 millimicrons (the exact position of the maximum and minimum of the gramicidin working standard should be determined for the particular instrument used). Subtract the absorptivity value at 247 millimicrons from the absorptivity value at 282 millimicrons.

$$\text{Absorptivity difference}=\frac{\text{Absorbance at 282 millimicrons}-\text{Absorbance at 247 millimicrons}}{0.0075}.$$

PART 148g—GRISEOFULVIN

Sec.
148g.1 Griseofulvin.
148g.2 Griseofulvin tablets.

AUTHORITY: §§ 148g.1 to 148g.2 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148g.1 Griseofulvin.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Griseofulvin is a white to pale-cream compound of a kind of griseofulvin or a mixture of two or more such compounds. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) It is nontoxic.

(iii) Its moisture content is not more than 1.0 percent.

(iv) Its melting point, after drying, is not less than 217° C. and not more than 220° C.

(v) Its specific rotation in dimethyl formamide at 25° C. is not less than +348° C. and not more than +364° C.

(vi) Its ultraviolet absorption spectrum in methyl alcohol is identical with that of the griseofulvin reference standard.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, toxicity, moisture, melting point, specific rotation, and identity.

(ii) Samples of the batch: 10 packages, each containing approximately equal portions of not more than 500 milligrams.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3)(ii) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Spectrophotometric assay—*(a) *Preparation of sample and standard solutions.* Accurately weigh approximately 35 milligrams each of the sample and standard, dissolve each in exactly 10.0 milliliters of dimethyl formamide, and mix well. Transfer 1.0-milliliter aliquots of the sample and standard to separate 500-milliliter volumetric flasks, bring to mark with distilled water, stopper, and mix well.

(b) *Procedure.* Adjust a suitable ultraviolet spectrophotometer to 100 percent light transmission with distilled water in the absorption cell. Determine the absorbance values for the sample and standard at the absorption peak at 294 millimicrons. If a recording spectrophotometer is used, record the ultraviolet absorption spectrum from 240 millimicrons to 320 millimicrons. If a nonrecording spectrophotometer is used, determine the absorbance value at the absorption peak. (The exact position of the peak should be determined for the particular instrument used.)

(c) *Calculations.* Determine the griseofulvin content of the sample as follows:

$$\text{Micrograms of griseofulvin per milligram}=\frac{A_1}{A_2}\times\frac{\text{Milligrams of standard}}{\text{Milligrams of sample}}\times\text{Potency of the standard in micrograms per milligram,}$$

where:

A_1 = Absorbance of sample at 294 millimicrons;
A_2 = Absorbance of standard at 294 millimicrons.

(ii) *Plate assay—*(a) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(b) *Culture media.* Medium I: Dextrose 40.0 grams, peptone 10.0 grams, and distilled water sufficient to make 1,000 milliliters. The pH should be 5.65±0.05 after sterilization.

Medium II: Dextrose 40.0 grams, peptone 10 grams, agar 15.0 grams, chloramphenicol U.S.P. 0.05 gram (activity), and distilled water sufficient to make 1,000 milliliters. The pH should be 5.65±0.05 after sterilization.

Medium III: Dextrose 40.0 grams, peptone 10.0 grams, agar 15.0 grams, chloramphenicol U.S.P. 0.05 gram (activity), and distilled water sufficient to make 1,000 milliliters. The pH should be 5.65 ±0.05 after sterilization. To each 100 milliliters of this agar at approximately 50° C. aseptically add 2 milliliters of a sterile solution of cycloheximide. The solution contains 10 milligrams per milliliter and is prepared by dissolving cycloheximide in distilled water. The solution is sterilized by filtering through a membrane filter having a porosity of 0.22 micron.

(c) *Working standard.* Dissolve about 50 milligrams of the working standard, accurately rectified, in sufficient *N,N*-dimethylforamide (DMF) to give a concentration of 1,000 micrograms per milliliter. This solution may be kept for 3 months under refrigeration.

(d) *Preparation of sample.* Dissolve an appropriate portion of the griseofulvin sample in DMF to give a convenient stock solution. Further dilute with DMF to give a concentration of 100 micrograms per milliliter. Then further dilute one part of this with 19 parts of 0.1*M* potassium phosphate buffer, pH 8.0, to give a final estimated concentration of 5.0 micrograms per milliliter of griseofulvin.

(e) *Preparation of test organism.* Grow Microsporum gypseum (ATCC 14683) for 3 weeks at 25° C. in four 3-liter Erlenmeyer flasks, each containing 200 milliliters of Medium I. Remove the floating mat from the flask with a sterile wire loop and place in a sterile blending jar. Aseptically add 200 milliliters of sterile distilled water and blend for approximately 30 seconds, allow to settle, then decant about 150 milliliters of the supernatant into 100-milliliter sterile, capped centrifuge tubes. Add the amount of distilled water equal to the amount removed each time, and repeat this process 4 to 5 times to assure maximum recovery of spores from the mycelial mat. Centrifuge the tubes containing the spores at 4,000 r.p.m. for 15 minutes and discard the supernatant. Wash the residual spores from all tubes, using a minimum of sterile distilled water (2 milliliters to 3 milliliters, per tube) and pool the washings into a sterile flask containing a few sterile glass

beads. (This procedure usually yields about 25 milliliters to 35 milliliters of spore suspension.) The spore suspension is stable for at least 2 months at 5° C.

(*f*) *Preparation of plates.* Prepare test plates using a 6-milliliter base layer of Medium II covered with a 4-milliliter seed layer of Medium III inoculated with varying volumes of spore suspension, e.g. 1 percent or 2 percent. Determine the proper percent of inoculum to be used by observation of the largest and clearest zones of inhibition given by 5 micrograms per milliliter of griseofulvin on the test plate. Add 6 milliliters of Medium II to a 20 millimeters x 100 millimeters Petri dish and allow to harden for use as a base layer. Cover the Petri dishes with sterile porcelain lids glazed on the outside. Add the proper amount (as previously determined) of the spore suspension to each 100 milliliters of Medium III which has been cooled to approximately 50° C. Mix the culture and agar thoroughly, and add 4 milliliters to each plate containing the uninoculated base agar. Tilt each plate back and forth to spread the inoculated agar evenly over the surface. When the agar has hardened, place six cylinders on the surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(*g*) *Standard curve.* Prepare the standard curve by diluting the 1,000 micrograms per milliliter griseofulvin solution to 64, 80, 100, 125, and 156 micrograms per milliliter in DMF. Then further dilute one part of each of these solutions with 19 parts of 0.1*M* potassium phosphate buffer, pH 8.0, to give final solutions of 3.2, 4.0, 5.0, 6.25, and 7.8 micrograms per milliliter. Use three plates for the determination of each point on the curve, except the 5.0 micrograms per milliliter solution, a total of 12 plates. On each of three plates fill three cylinders with the 5.0 micrograms per milliliter solution and the other three cylinders with the concentration of the standard curve under test. There will be thirty-six 5.0 micrograms per milliliter determinations and 9 determinations each for each of the other points on the curve. Incubate the plates for at least 48 hours at 30° C., and measure the diameters of the circles of inhibition. (Plates may be incubated longer if zones are indistinct.) Average the 36 readings of the 5.0 micrograms per milliliter concentration and the readings of the other concentrations for each set of three plates. The average of the 36 readings of the 5.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 5.0 micrograms per milliliter reading for that set of 3 plates were the same as the correction point. Thus, if in correcting the 4.0 micrograms per milliliter concentration, the average of the 36 readings of the 5.0 micrograms per milliliter concentration is 16.6 millimeters, and the average of the 5.0 micrograms per milliliter concentration of this set of 3 plates is 16.4 millimeters, the correction is plus 0.2 millimeter. If the average reading of the 4.0 micrograms per milliliter concentration of these same three plates is 14.8, the corrected value is then 15.0 millimeters. Plot these corrected values, including the average of the 5.0 micrograms per milliliter concentration on semilogarithmic paper, placing the griseofulvin concentrations on the logarithmic scale and the diameters of the zones on the arithmetic scale. Draw the line of best fit by inspection or by calculation as described in § 141a.1(h) of this chapter.

(*h*) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 5.0 microgram per milliliter solution and three cylinders with the sample solution to be tested, alternating standard and sample. Incubate the plates for at least 48 hours at 30° C. and measure the diameter of each circle of inhibition. To calculate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on each set of plates. If the sample gives larger average zone size than the standard, add the difference between them to the 5.0 micrograms per milliliter zone. If the average sample value is lower than the standard value, subtract the difference between them from the 5.0 micrograms per milliliter value on the curve. Read from the standard curve the concentration corresponding to the corrected zone size.

(2) *Toxicity.* Administer orally, by means of a cannula or other suitable device, to each of 5 mice within the weight range of 18 grams to 25 grams, 1.0 milliliter of a suspension of the drug containing 100 milligrams per milliliter in 10 percent aqueous solution of acacia. If no animal dies within 48 hours, the sample is nontoxic. If one or more animals die within 48 hours, repeat the test with 5 unused mice weighing 20 grams (±0.5 gram) each; if all animals survive the repeat test the sample is nontoxic.

(3) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(4) *Melting point.* Proceed as directed in the U.S.P.

(5) *Specific rotation.* Accurately weigh approximately 25 milligrams of the sample in a 25-milliliter glass-stoppered volumetric flask and dissolve in about 15 milliliters of dimethyl formamide. Bring to volume with dimethyl formamide, stopper, and mix well. Transfer to a 200-millimeter optical rotation tube. Determine the angular rotation in a suitable polarimeter, using a wavelength of 5893 Angstrom units, and calculate the specific rotation.

(6) *Identity.* Prepare solutions of each of the griseofulvin sample and of the reference standard containing 7 micrograms per milliliter in methyl alcohol. Record the ultraviolet absorption spectrum from 240 millimicrons to 320 millimicrons. The curves shall be similar, and each shall have a maximum at 291 millimicrons, ±2 millimicrons, and a minimum at 270 millimicrons, ±2 millimicrons.

§ 148g.2 Griseofulvin tablets.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Griseofulvin tablets are tablets composed of griseofulvin, with or without one or more suitable diluents, lubricants, and binders. Each tablet contains not less than 250 milligrams of griseofulvin. The moisture content is not more than 3.0 percent. Tablets not exceeding 15 millimeters in diameter, or not intended only for preparing solutions, shall disintegrate within 1 hour. The griseofulvin used conforms to the requirements of § 148g.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Requests for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The griseofulvin used in making the batch for potency, toxicity, melting point, specific rotation, and identity.

(*b*) The batch for potency, moisture, and disintegration time.

(ii) Samples required:

(*a*) Griseofulvin used in making the batch: 10 packages, each containing approximately equal portions of not less than 300 milligrams.

(*b*) The batch:

(*1*) For all tests except disintegration time: Minimum of 30 tablets.

(*2*) For disintegration time: Six tablets.

(*c*) In case of an initial request for certification, each other substance used in making the batch: One package of each containing not less than 5 grams.

(4) *Fees.* $0.75 for each tablet in the samples submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $3.00 for all tablets submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph; $4.00 for each package submitted in accordance with subparagraph (3)(ii)(*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Chemical assay.* Proceed as directed in § 148g.1(b)(1)(i), except prepare the sample for assay by reducing to a fine powder not less than 10 average-weight tablets. Weigh accurately an amount of the fine powder equivalent to approximately 175 milligrams of griseofulvin. Transfer to a 50-milliliter volumetric flask. Add about 35 milliliters of dimethyl formamide, stopper, and shake approximately 30 minutes. Dilute to volume with dimethyl formamide and mix well. Centrifuge an aliquot (about 10 milliliters to 15 milliliters). Transfer a 1.0-milliliter aliquot of the clear supernatant to a 500-milliliter volumetric flask and dilute to volume with distilled water.

(ii) *Plate assay.* Powder the tablets as described in subdivision (i) of this subparagraph, and proceed as directed in § 148g.1(b)(1)(ii). The average content of griseofulvin is satisfactory if it is not less than 90 percent and not more than 115 percent of the number of milligrams that it is represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.5(a) of this chapter.

(3) *Disintegration time.* Proceed as directed in § 141a.9(c) of this chapter. The tablets are satisfactory if they disintegrate within 1 hour.

PART 148h—KANAMYCIN SULFATE

Sec.
148h.1 Kanamycin sulfate.
148h.2 Kanamycin sulfate aqueous injection.
148h.3 Kanamycin sulfate capsules.

AUTHORITY: §§ 148h.1 to 148h.3 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148h.1 Kanamycin sulfate.

(a) *Requirements for certification—*(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate is the crystalline sulfate salt of a kind of kanamycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency on the anhydrous basis is not less than 750 micrograms per milligram.

(ii) It is sterile.

(iii) It is nontoxic.

(iv) It is nonpyrogenic.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 6 percent.

(vii) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(viii) It gives a positive identity test for kanamycin.

(ix) It contains not more than 5.0 percent kanamycin B.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, toxicity, pyrogens, histamine, moisture, pH, identity, crystallinity, and kanamycin B content.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*b*) For sterility testing: 10 packages, each containing approximately 500 milligrams.

(4) *Fees.* $10.00 for each container submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay—*(1) *Potency—*(i) *Cylinders (cups).* Use cylinders described under § 141a.1(a) of this chapter.

(ii) *Culture medium.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. Make nutrient agar for the base and seed layers as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Glucose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.8 to 8.0 after sterilization.

(iii) *Working standard.* Dissolve a suitable quantity of the working standard accurately weighed, in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a convenient stock solution. The stock solution, when stored at a temperature of approximately 15° C., or less, may be used for a period not exceeding 1 month.

(iv) *Preparation of sample.* Dissolve a suitable quantity, accurately weighed, in 0.1*M* potassium phosphate buffer, pH 8.0, to give a convenient stock solution. Further dilute volumetrically in 0.1*M* potassium phosphate buffer, pH 8.0, to make a solution containing 5.0 micrograms of kanamycin base per milliliter (estimated).

(v) *Preparation of test organism.* The test organism is Staphylococcus aureus (ATCC 6538P) which is maintained on agar described in subdivision (ii) of this subparagraph, except that the pH after sterilization is 6.5 to 6.6. From a stock slant, inoculate a Roux bottle containing the same agar and incubate for 24 hours at 32° C. to 35° C. Wash the growth from the agar surface with 50 milliliters of sterile sodium chloride solution. If an aliquot of this bulk suspension, when diluted 1:20 with sterile sodium chloride solution, gives 25 percent light transmission in a suitable photoelectric colorimeter equipped with a filter having a wavelength of 580 millimicrons, it is satisfactory for use. It may be necessary to adjust the bulk suspension by dilution, so that an aliquot of the adjusted suspension diluted 1:20 gives 25 percent light transmission. (The adjusted bulk suspension only, and not the 1:20 dilution of it, is used in preparing the seed layer.) The bulk suspension may be stored for 1 week under refrigeration. Add 0.4 milliliter of the adjusted bulk suspension to 100 milliliters of agar described in subdivision (ii) of this subparagraph, which has been melted and cooled to 48° C.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in subdivision (ii) of this subparagraph to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates and allow to harden. Use the plates the same day they are prepared. Add 4.0 milliliters of the inoculum prepared as described in subdivision (v) of this subparagraph to each plate, tilting the plates back and forth to spread the inoculated agar evenly over the surface.

(vii) *Standard curve.* Prepare daily in 0.1*M* potassium phosphate buffer, pH 8.0, from the stock solution described in subdivision (iii) of this subparagraph, concentrations of 3.2, 4.0, 5.0, 6.25, and 7.81 micrograms per milliliter of solution. A total of 12 plates is used in the preparation of the standard curve, three plates for each solution except the 5.0 micrograms per milliliter solution. The latter concentration is used as the reference point and is included on each plate. On each of three plates, fill three cylinders with the 5.0 microgram per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 5-microgram determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 5.0 micrograms per milliliter concentration and the readings of the concentration tested for each set of three plates and average also all 36 readings of the 5.0 micrograms per milliliter concentration. The average of the 36 readings of the 5.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 5.0 micrograms per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 4.0-microgram concentration, the average of the 36 readings of the 5.0 micrograms per milliliter concentration is 16.5 millimeters and the average of the 5.0 micrograms per milliliter concentration of this set of three plates is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 4.0 micrograms per milliliter concentration of these same three plates is 15.9 millimeters, the corrected value is then 16.1 millimeters. Plot these corrected values, including the average of the 5.0 micrograms per milliliter concentration, on 2-cycle semilog paper, using the concentration in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5}.$$

$$H=\frac{3e+2d+c-a}{5}.$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 5.0 micrograms per milliliter standard;

a, b, d, e = Corrected average values for the 3.2, 4.0, 6.25, and 7.81 micrograms per milliliter solutions, respectively.

Plot the values obtained for L and H and connect wtih a straight line.

(viii) *Assay.* Place six cylinders on the inoculated agar surface in each Petri dish prepared as described in subdivision (vi) of this subparagraph, so that they are at approximately 60° intervals on 2.8-centimeter radius. Use three plates for each sample. Fill three cylinders on each plate with the 5.0 micrograms per milliliter standard and three cylinders with the 5.0 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate plates for 16 hours to 18 hours at 32° C. to 35° C., and measure the diameter of each circle of inhibition.

(ix) *Estimation of potency.* Average the zone readings of the standard and average the zone readings of the sample on the three plates used. If the sample gives larger average zone size than the

average of the standard, add the difference between them to the 5.0-microgram zone sizes of the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 5.0-microgram value on the curve. From the curve, read the potencies corresponding to these corrected values of zone sizes.

(2) *Sterility.* Using 500 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, using 0.5 milliliter of a solution prepared by diluting the sample to approximately 2.0 milligrams (activity) per milliliter with sterile distilled water.

(4) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using a test dose of 1.0 milliliter per kilogram of an aqueous solution containing 10 milligrams per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using as a test dose 0.6 milliliter per kilogram of an aqueous solution containing 5 milligrams per milliliter.

(6) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a solution containing 10 milligrams per milliliter.

(8) *Identity.* Dissolve about 10 milligrams of kanamycin sulfate in 1 milliliter of water, and add 1 milliliter of a 1:500 solution of triketohydrindene hydrate in normal butyl alcohol, then add 0.5 milliliter of pyridine. Heat on a steam bath for 5 minutes, and add 10 milliliters of water; a deep-purple color is produced.

(9) *Kanamycin B content*—(i) *Cylinders (cups).* Use cylinders described under § 141a.1(a) of this chapter.

(ii) *Culture medium.* Use ingredients that conform to the standards prescribed by the U.S.P. or N.F. Make agar for the base and seed layers as follows:

Peptone	6.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Agar	15.0 gm.

ph 7.8 to 8.0 after sterilization.

(iii) *Working standard.* Dissolve a suitable quantity of the kanamycin sulfate reference standard, accurately weighed, in 0.1*M* potassium phosphate buffer, pH 8.0, to give a concentration equivalent to 1.0 milligram of kanamycin per milliliter.

(iv) *Preparation of sample.* To 50 milligrams, accurately weighed, of kanamycin sulfate in a suitable container (such as a 7.5-milliliter serum vial) add 2.5 milliliters of 6*N* hydrochloric acid, and tightly close the container. Heat in a water bath at 100° C. for 1 hour, and cool. Add 2 milliliters of 6*N* sodium hydroxide, then dilute with sterile 0.1*M* potassium phosphate buffer, pH 8.0, to obtain a concentration of the equivalent of 1 microgram of kanamycin B per milliliter (estimated).

(v) *Preparation of test organism.* Use Bacillus subtilis (ATCC 6633) maintained on the peptone agar described in subdivision (ii) of this subparagraph. From a stock slant, inoculate a Roux bottle containing approximately 300 milliliters of the same agar and incubate for 24 hours at 32° C. to 35° C. Wash the growth from the agar surface with 50 milliliters of sterile U.S.P. saline (T.S.). Determine by appropriate tests the quantity of this viable spore suspension to be added to each 100 milliliters of peptone agar to give sharp, clear zones of inhibition.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in this subparagraph to each Petri dish (20 millimeters×100 millimeters). Distribute the agar evenly in the plates and allow to harden. Use the plates the same day they are prepared. Add 4.0 milliliters of the fresh daily inoculum described in subdivision (iv) of this subparagraph to each plate, tilting the plates back and forth to spread the inoculated agar evenly over the surface.

(vii) *Standard curve.* Prepare on the day of testing in 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0, from the standard stock solution, sufficient volumes of the following concentrations: 0.64, 0.8, 1.0, 1.25, and 1.56 micrograms per milliliter. The 1.0 microgram per milliliter solution is the reference point of the standard curve. On each of three plates fill three cylinders with the 1.0 microgram per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 1.0-microgram determinations and nine determinations for each of the other points on the curve. After the plates have incubated, read the diameters of the circles of inhibition. Average the readings of the 1.0 microgram per milliliter concentration and the readings of the concentration test for each set of three plates and average also all 36 readings of the 1.0 microgram per milliliter concentration. The average of the 36 readings of the 1.0 microgram per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 1.0 microgram per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 0.8-microgram concentration, the average of the 36 readings of the 1.0 microgram per milliliter concentration is 16.5 millimeters and the average of the 1.0 microgram per milliliter concentration of this set of three plates is 16.3 millimeters, the correction is +0.2 millimeter. If the average readings of the 0.8 microgram per milliliter concentration of these same three plates is 15.9 millimeters, the corrected value is 16.1 millimeters. Plot these corrected values, including the average of the 1.0 microgram per milliliter concentration, on 2-cycle semilogarithmic paper, using the concentration in micrograms per milliliter as the ordinate and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Calculated zone diameter for the lowest concentration of the standard curve;

H = Calculated zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 1.0 microgram per milliliter standard;

a, b, d, e = Corrected average values for the 0.64, 0.8, 1.0, 1.25, and 1.56 micrograms per milliliter solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay.* Place six cylinders on the inoculated agar surface in each Petri dish prepared as described in subdivision (vi) of this subparagraph, so that they are at approximately 60° intervals on a 2.8-centimeter radius. Use three plates for each sample. Fill three cylinders on each plate with the 1.0 microgram per milliliter standard and three cylinders with the 1.0 microgram (estimated) per milliliter sample, alternating standard and sample. Incubate plates for 16 hours to 18 hours at 32° C. to 35° C., and measure the diameter of each circle of inhibition.

(ix) *Estimation of kanamycin B content.* Average the zone readings of the standard and average the zone readings of the sample on the three plates used. If the sample gives larger average zone size than the average of the standard, add the difference between them to the 1.0-microgram zone sizes of the standard curve. If the average value is lower than the standard value, subtract the difference between them from the 1.0-microgram value on the curve. From the curve, read the kanamycin potencies corresponding to these corrected values of zone sizes. Multiply the observed potency by 100 and divide by 126, to obtain a value representing the potency in terms of the milligram equivalent of kanamycin B sulfate. The calculated amount of kanamycin B sulfate is not more than 5 percent of the content of kanamycin sulfate found in subparagraph (1)(i) of this paragraph.

(10) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

§ 148h.2 Kanamycin sulfate aqueous injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate for aqueous injection is an aqueous solution of kanamycin sulfate with suitable and harmless buffer substances and preservatives. Its potency is not less than 250 milligrams per milliliter. It is sterile. It is nontoxic. It is nonpyrogenic. It contains no histamine nor histamine-like substances. Its pH is not less than 3.5 and not more than 5.0. The kanamycin sulfate used conforms to the requirements of § 148h.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or the N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* (i) In addition to the requirements prescribed by § 148.3 of this chapter, the labeling of each package shall bear a warning to the effect that older patients and patients receiving a total dose of more than 20 grams of the drug should be carefully observed for signs of eighth-nerve damage. In patients with impaired kidney function or with prerenal azotemia, the risk of severe ototoxic reaction that may result in permanent deafness is sharply increased.

(ii) The expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The kanamycin sulfate used in making the batch for potency, identity, crystallinity, and kanamycin B content.

(*b*) The batch for potency, sterility, toxicity, pyrogens, histamine, and pH.

(ii) Samples required:

(*a*) The kanamycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: Minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $4.00 for each container in the samples submitted in accordance with subparagraph (3)(ii) (*a*), (*b*)(*1*), and (*c*) of this paragraph; $10.00 for all containers submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148h.1(b)(1), except prepare the sample for assay by introducing a representative aliquot of the solution equivalent to one dose into a 100-milliliter volumetric flask and dilute to mark with 0.1*M* potassium phosphate buffer, pH 8.0. Mix thoroughly and make to proper estimated dilution in pH 8.0 buffer. The average potency is satisfactory if the immediate containers contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Sterility.* Using the entire contents of single-dose containers or the equivalent of approximately 500 milligrams from each multiple-dose container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Toxicity.* Proceed as directed in § 148h.1(b)(3).

(4) *Pyrogens.* Proceed as directed in § 148h.1(b)(4).

(5) *Histamine.* Proceed as directed in § 148h.1(b)(5).

(6) *pH.* Proceed as directed in § 141a.5(b) of this chapter using undiluted solution.

§ 148h.3 Kanamycin sulfate capsules.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Kanamycin sulfate capsules are composed of crystalline kanamycin sulfate, with or without one or more suitable and harmless buffer substances, vegetable oils, preservatives, diluents, binders, lubricants, colorings, and flavorings, enclosed in gelatin capsules. Each capsule contains the equivalent of not less than 500 milligrams of kanamycin base. The moisture content is not more than 6 percent. The crystalline kanamycin sulfate used conforms to the requirements of § 148h.1(a)(1) (i), (iii), (vii), (viii), and (ix). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) The kanamycin sulfate used in making the batch for potency, toxicity, pH, crystallinity, kanamycin B content, and identity.

(*b*) The batch for potency and moisture.

(ii) Samples required:

(*a*) Kanamycin sulfate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch: Minimum of 30 capsules.

(*c*) In case of an initial request for certification, each other ingredient used in making the batch: One package of each containing approximately 5 grams.

(4) *Fees.* $0.75 for each capsule in the sample submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph; $4.00 for each immediate container submitted in accordance with subparagraph (3)(ii) (*a*) and (*c*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Use either of the following methods:

(i) *Plate assay.* Proceed as directed in § 148h.1(b)(1), except prepare the sample for assay as follows: Place three capsules in a glass blending jar containing 500 milliliters of 0.1*M* potassium phosphate buffer, pH 7.8 to 8.0. Using a high-speed blender, blend 3 minutes to 5 minutes, and then remove an aliquot and make to the proper estimated dilution in pH 8.0 buffer.

(ii) *Turbidimetric assay.* Proceed as directed in the U.S.P.

The average potency of the capsules is satisfactory if they contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter.

PART 148l—[RESERVED]

PART 148m—OLEANDOMYCIN

Sec.

148m.1 Oleandomycin phosphate.
148m.2 [Reserved]
148m.3 Oleandomycin phosphate for injection.

AUTHORITY: §§ 148m.1 to 148m.3 issued under sec. 507, 59 Stat. 463, as amended 76 Stat. 785, 786, 787; 21 U.S.C.A. 357.

§ 148m.1 Oleandomycin phosphate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oleandomycin phosphate is the crystalline phosphate salt of a kind of oleandomycin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 775 micrograms of oleandomycin base per milligram.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) It is nontoxic.

(v) It contains no histamine nor histamine-like substances.

(vi) Its moisture content is not more than 5 percent.

(vii) Its pH in a solution containing 100 milligrams per milliliter is not less than 3.0 and not more than 6.0.

(viii) Its sulfated ash content is not more than 1 percent.

(ix) Its heavy metals content is not more than 30 parts per million.

(x) It gives a positive identity test for oleandomycin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3(b) of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, toxicity, histamine, moisture, pH, crystallinity, ash content, heavy metals, and identity.

(ii) Samples on the batch:

(*a*) For all tests except sterility: 10 packages, each containing equal portions of not less than 500 milligrams.

(*b*) For sterility testing: 10 packages each containing not less than 500 milligrams.

(4) *Fees.* $10.00 for each container submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for all samples submitted in accordance with subparagraph (3)(ii)(*b*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency*—(i) *Cylinders (cups).* Use cylinders described in § 141a.1(a) of this chapter.

(ii) *Culture media*—(*a*) Make nutrient agar for the seed and base layers as follows:

Peptone	6.0 gm.
Pancreatic digest of casein	4.0 gm.
Yeast extract	3.0 gm.
Beef extract	1.5 gm.
Glucose	1.0 gm.
Agar	15.0 gm.
Distilled water, q.s.	1,000.0 ml.

pH 7.8 to 8.0 after sterilization.

(*b*) Use the nutrient agar described in subsection (*a*) of this subdivision for maintaining the test organism, except that its pH after sterilization is 6.5 to 6.6.

(iii) *Working standard.* Dissolve a suitable weighed quantity (usually 25 milligrams or less) of the working standard in 2 milliliters of ethyl alcohol, then add sufficient 0.1*M* potassium phosphate

buffer, pH 8.0, to give a concentration of 1,000 micrograms of oleandomycin base per milliliter. This stock solution may be kept in the refrigerator for 3 days.

(iv) *Preparation of sample.* Dissolve the sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0, to give a convenient stock solution. Further dilute in 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 5.0 micrograms of oleandomycin base per milliliter (estimated).

(v) *Preparation of test organism.* The test organism is Staphylococcus epidermidis (ATCC 12228), which is maintained on slants of agar described in subdivision (ii) (*b*) of this subparagraph. Wash the organism from the agar slant with 3 milliliters of sterile sodium chloride solution onto a large agar surface such as that provided by a Roux bottle containing 300 milliliters of the agar described in subdivision (ii) (*b*) of this subparagraph. Spread the suspension of organisms over the entire agar surface with the aid of sterile glass beads. Incubate for 24 hours at 32° C. and then wash the resulting growth from the agar surface with about 50 milliliters of sterile sodium chloride solution. Standardize the suspension so that a 1:25 dilution of it will give 25 percent light transmission, using a suitable photoelectric colorimeter with a 580-millimicron filter and a 13-millimeter diameter test tube as an absorption cell. Run test plates to determine the quantity of the bulk suspension (usually 0.16 milliliter) that should be added to each 100 milliliters of agar to give clear, sharp zones of inhibition of appropriate size.

(vi) *Preparation of plates.* Add 21 milliliters of the agar prepared as described in subdivision (ii) (*a*) of this subparagraph to each Petri dish (20 millimeters x 100 millimeters). Distribute the agar evenly in the plates and allow it to harden. Use the plates the same day they are prepared. Melt a sufficient amount of the agar described in subdivision (ii) (*a*) of this subparagraph, cool to 48° C., add the proper amount of the test organism as described in subdivision (v) of this subparagraph, and mix thoroughly. Add 4 milliliters of this inoculated agar to each Petri dish. Distribute the agar evenly in the plates, cover with porcelain covers glazed on the outside, and allow to harden. After the agar has hardened, place 6 cylinders on the agar surface so that they are at approximately 60° intervals on a 2.8-centimeter radius.

(vii) *Standard curve.* Prepare the daily standard curve by further diluting the 1,000 micrograms per milliliter stock solution in 0.1*M* potassium phosphate buffer, pH 8.0, to obtain concentrations of 3.2, 4.0, 5.0, 6.25 and 7.80 micrograms per milliliter. Use three plates for the determination of each point on the curve, except the 5.0 micrograms per milliliter concentration, a total of 12 plates. On each of three plates, fill three cylinders with the 5.0 micrograms per milliliter standard and the other three cylinders with the concentration under test. Thus, there will be thirty-six 5-microgram determinations and nine determinations for each of the other points on the curve. After incubation, read the diameters of the circles of inhibition in the plates. Average the readings of the 5.0 micrograms per milliliter concentration and the readings of the point tested for each set of three plates and average also all 36 readings of the 5.0 micrograms per milliliter concentration. The average of the 36 readings of the 5.0 micrograms per milliliter concentration is the correction point for the curve. Correct the average value obtained for each point to the figure it would be if the 5.0 micrograms per milliliter reading for that set of three plates were the same as the correction point. Thus, if in correcting the 4.0-microgram concentration, the average of the 36 readings of the 5.0 microgram concentration were 20 millimeters, and the average of the 5.0-microgram concentration of this set of three plates were 19.8 millimeters, the correction would be +0.2 millimeter. If the average reading of the 4.0-microgram concentration of these same three plates were 19.0 millimeters, the corrected value would be 19.2 millimeters. Plot these corrected values, including the average of the 5.0 micrograms per milliliter concentration, on 2-cycle semilog paper, using the concentrations in micrograms per milliliter as the ordinate (the logarithmic scale) and the diameter of the zone of inhibition as the abscissa. Draw the standard curve through these points, either by inspection or by means of the following equations:

$$L=\frac{3a+2b+c-e}{5},$$

$$H=\frac{3e+2d+c-a}{5},$$

where:

L = Corrected zone diameter for the lowest concentration of the standard curve;

H = Corrected zone diameter for the highest concentration of the standard curve;

c = Average zone diameter of 36 readings of the 5.0 micrograms per milliliter standard;

a, b, d, e = Corrected average values for 3.2, 4.0, 6.25, and 7.81 micrograms per milliliter standard solutions, respectively.

Plot the values obtained for L and H and connect with a straight line.

(viii) *Assay.* Use three plates for each sample. Fill three cylinders on each plate with the standard 5.0 micrograms per milliliter solution and three cylinders with the 5.0 micrograms per milliliter (estimated) sample, alternating standard and sample. Incubate all plates, including those containing the standard curve at 32° C.–35° C. overnight, and measure the diameter of each circle of inhibition. To estimate the potency of the sample, average the zone readings of the standard and the zone readings of the sample on the three plates used. If the sample gives a larger zone size than the average of the standard, add the difference between them to the 5.0 micrograms per milliliter zone on the standard curve. If the average sample value is lower than the standard value, subtract the difference between them from the 5.0 micrograms per milliliter value on the curve, and read the potencies corresponding to these corrected values of zone sizes.

(2) *Sterility.* Using 500 milligrams from each container tested, proceed as directed in § 141a.2 of this chapter, except that neither penicillinase nor the control tube is used in the test for bacteria.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using sterile, pyrogen-free saline T.S. as a diluent, and inject 1.0 milliliter per kilogram of a solution containing 10 milligrams (activity) per milliliter.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except inject 0.5 milliliter of a solution in sterile saline T.S. containing 8 milligrams of oleandomycin base per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using saline T.S. as a diluent, and injecting 1.0 milliliter of a solution containing 3 milligrams activity per milliliter.

(6) *Moisture.* Proceed as directed in § 141a.26(e) of this chapter, except use a sample of approximately 1 gram, accurately weighed.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using a solution containing 100 milligrams (activity) of oleandomycin per milliliter.

(8) *Crystallinity.* Proceed as directed in § 141a.5(c) of this chapter.

(9) *Ash content.* Proceed as directed in § 141e.401(g) of this chapter.

(10) *Heavy metals*—(i) *Preparation of reagents*—(*a*) *Ammonia, T.S.* Prepare an aqueous solution containing not less than 9 grams and not more than 10 grams of NH_3 per 100 milliliters.

(*b*) *Diluted acetic acid.* Dilute 60.0 milliliters of glacial acetic acid with sufficient water to give a solution of 1,000 milliliters.

(*c*) *Hydrogen sulfide, T.S.* Prepare a saturated solution of hydrogen sulfide by passing H_2S into cold water for a sufficient time. It is suitable if it produces at once a copious precipitate when added to an equal volume of 1*N* ferric chloride. Prepare a fresh solution of hydrogen sulfide T.S. each time a metals test is performed.

(*d*) *Lead nitrate stock solution.* Dissolve 159.8 milligrams of lead nitrate with 100 milliliters of water to which has been added 1 milliliter of nitric acid, and adjust with water to a volume of 1,000 milliliters. Prepare and store this solution in glass containers free from soluble lead salts.

(*e*) *Standard lead solution.* Dilute a 10.0-milliliter aliquot of the lead nitrate stock solution to 100 milliliters with water. This solution must be freshly prepared each time a heavy metals test is performed. One milliliter of this standard lead solution represents a level of 10 parts per million of lead.

(ii) *Preparation of the sample.* Use the sulfated ash obtained in the ash content test, or if necessary ash a separate weighing of 1.0 gram, accurately weighed, or 0.5 gram if the heavy metals limit is more than 30 parts per million. If a separate weighing of the substance is made, carefully ignite in a porcelain crucible at a low temperature until thoroughly charred. The crucible may be loosely covered with a porcelain lid during the charring. Add to the contents of the crucible 2 milliliters of nitric

acid and 5 drops of sulfuric acid, and cautiously heat until white fumes are evolved, then ignite, preferably in a muffle furnace at 500° C. to 600° C. for 3 hours or until the carbon is all burned off. After cooling, add 2 milliliters of hydrochloric acid, and slowly evaporate to dryness on a steam bath. Moisten the residue with 1 drop of hydrochloric acid, add 10 milliliters of hot water, and digest for 2 minutes. Add, dropwise, ammonia T.S. until the solution is just alkaline to litmus paper, then add, dropwise, diluted acetic acid until the solution is slightly acid to litmus paper, and then add an excess of 2 milliliters of the diluted acetic acid. Filter if necessary, wash the crucible and the filter with about 10 milliliters of water, and dilute to exactly 25 milliliters.

(iii) *Procedure.* Place in a 50-milliliter Nessler tube 2 milliliters of diluted acetic acid and exactly the quantity of standard lead solution containing the lead equivalent of the heavy metals limit specified for the substance to be tested and add water to make 25 milliliters. Transfer the 25-milliliter solution of the sample, subdivision (ii) of this subparagraph, to a 50-milliliter Nessler tube, and add 10 milliliters of hydrogen sulfide T.S. to each tube, mix, allow to stand for 10 minutes, and view downward over a white surface; the color of the solution of the sample should be no darker than the standard that contains the lead equivalent of the heavy metals limit of the test.

(11) *Identity.* Dissolve about 10 milligrams of oleandomycin phosphate in 5 milliliters of hydrochloric acid and heat the solution in a water bath; a greenish-yellow color is produced.

§ 148m.3 Oleandomycin phosphate for injection.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Oleandomycin phosphate for injection is oleandomycin phosphate, with or without one or more suitable and harmless buffer substances and preservatives. Each vial contains not less than 500 milligrams of oleandomycin activity as the phosphate salt. It is sterile. It is nonpyrogenic. It is nontoxic. It contains no histamine nor histamine-like substances. Its moisture content is not more than 5.0 percent. The pH of a reconstituted solution containing 5 milligrams of oleandomycin activity per milliliter is not less than 3.0 and not more than 5.0. The oleandomycin phosphate used conforms to the requirements prescribed by § 148m.1(a)(1). Each other substance used, if its name is recognized in the U.S.P. or N.F., conforms to the standards prescribed therefor by such official compendium.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 148.3 of this chapter. Its expiration date is 12 months.

(3) *Request for certification.* In addition to the requirements of § 148.4 of this chapter, each such request shall contain:

(i) Results of tests and assays on:

(*a*) Oleandomycin phosphate used in making the batch for potency, identity, and crystallinity.

(*b*) The batch for potency, sterility, toxicity, pyrogens, histamine, moisture, and pH.

(ii) Samples required:

(*a*) Oleandomycin phosphate used in making the batch: 10 packages, each containing approximately 500 milligrams.

(*b*) The batch:

(*1*) For all tests except sterility: Minimum of 10 immediate containers.

(*2*) For sterility testing: 10 immediate containers.

(4) *Fees.* $4.00 for each container in the sample submitted in accordance with subparagraph (3)(ii)(*a*) of this paragraph; $10.00 for each container submitted in accordance with subparagraph (3)(ii)(*b*)(*1*) of this paragraph; $10.00 for all immediate containers submitted in accordance with subparagraph (3)(ii)(*b*)(*2*) of this paragraph.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 148m.1(b)(1), except prepare the sample for assay as follows: Reconstitute the drug in the immediate containers as directed in the labeling and remove with a syringe and needle equivalent to one dose. Dilute the one-dose sample volumetrically in 0.1*M* potassium phosphate buffer, pH 8.0, to give a final concentration of 5.0 micrograms of oleandomycin base per milliliter (estimated). The average potency is satisfactory if the immediate containers contain not less than 90 percent and not more than 115 percent of the number of milligrams they are represented to contain.

(2) *Sterility.* Proceed as directed in § 141a.2 of this chapter, using 500 milligrams (activity) of oleandomycin phosphate from each container tested.

(3) *Pyrogens.* Proceed as directed in § 141a.3 of this chapter, using 1.0 milliliter per kilogram of a solution containing 10 milligrams (activity) per milliliter in sterile, pyrogen-free saline T.S.

(4) *Toxicity.* Proceed as directed in § 141a.4 of this chapter, except use sterile saline T.S. as a diluent, and inject 0.5 milliliter of a solution containing 8 milligrams of oleandomycin activity per milliliter.

(5) *Histamine.* Proceed as directed in § 141b.105 of this chapter, using sterile saline T.S. as a diluent, and inject 1 milliliter of a solution containing 3 milligrams (activity) of oleandomycin per milliliter.

(6) *Moisture.* Proceed as directed in § 141a.5(a) or § 141a.26(e) of this chapter.

(7) *pH.* Proceed as directed in § 141a.5(b) of this chapter, using an aqueous solution containing 5 milligrams of oleandomycin base per milliliter.

(8) *Identity.* Dissolve about 10 milligrams of oleandomycin phosphate in 5 milliliters of hydrochloric acid and heat the solution in a water bath; a greenish-yellow color is produced.

All interested persons are invited to present their views in writing regarding this proposal to the Hearing Clerk, Department of Health, Education, and Welfare, Washington 25, D.C., within 30 days from the date of publication of this notice in the FEDERAL REGISTER. Such views and comments should be filed preferably in quintuplicate.

Proposals for the certification of various other antibiotic drugs will be published in the immediate future.

Dated: April 24, 1963.

GEO. P. LARRICK,
Commissioner of Food and Drugs.

[F.R. Doc. 63-4620; Filed, May 1, 1963; 8:45 a.m.]